

**PHARMACOGNOSTICAL, PHYTOCHEMICAL INCLUDING
ISOLATION OF PHENOLIC RICH FRACTION, HPTLC
ANALYSIS AND PHARMACOLOGICAL STUDIES ON THE
LEAVES OF *Xanthium strumarium* (linn)**



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MASTER OF PHARMACY IN PHARMACOGNOSY

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BY

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CHAPTER 1

INTRODUCTION

Herbal drug constitute a major part in all the traditional system of medicine. Herbal medicine is a triumph of popular therapeutic diversity. Plants have been used for medicine since time immemorial because they have fitted immediate personal need and are easily accessible and inexpensive.¹

The Indian sub continent is enriched by a variety of flora both aromatic and medicinal plants. This is due to the wide diversity of agro-climatic conditions ranging from deserts to swamplands. India is virtually considered as the herbarium of the world and has been called the “BOTANICAL GARDEN OF THE WORLD”. This botanical wealth constitute more than 2200 species of medicinal and essential oil containing plants.³

HERBAL PHARMACOVIGILANCE¹

Pharmacovigilance is the science and activities relating to the detection, assessment, understanding and prevention of adverse effect or any other possible drug related problems. Herbal pharmacovigilance is the need of the day. Though in India we have so many ancient systems of medicine like Ayurveda, Siddha, Unani etc, which are being practiced from time immemorial it's a pity that proper pharmacovigilance is lacking. There is no proper evidence been started in many countries. By analog with conventional pharmacovigilance, herbal pharmacovigilance aims at the detection of serious adverse reactions, at the quantification of their incidence, and at the identification of contributive and modifying factors.

Proper pharmacovigilance of all the phyto-therapeutic claims on the herbals is essential for the treatment and evaluation of the herbal health care around the globe. Thus, herbal pharmacovigilance is not a negative tool but a neutral one, especially when it identifies a new serious herbal health risk. Pharmacovigilance can also be reassuring, however, by providing evidence that certain herbal health risks are absent or negligibly small. Thus, it can help booster one of the main features of phytotherapeutics, namely their relative safety when compared to conventional pharmaceuticals.

The use of plants for medicines around the world still vastly exceeds the use of modern synthetic drugs. Such activity is not completely dismissed in scientific society and plants are also appreciated in pharmaceutical research as the major resource for new medicines and a growing body of medical literature supports the clinical efficacy of herbal treatments. Even where traditional use has largely died out in developed countries, there is an increasing yearning for a new deal in healthcare in which the old remedies feature strongly.

Natural products will continue to be important in three areas of drug discovery

- As targets for production by biotechnology
- As a source of new lead compounds of novel chemical structure and
- As the active ingredients of useful treatments derived from traditional systems of medicine.

The recent analysis confirmed that among the top 20 brands of drugs for the treatment of various ailments about 40% are natural products and derived natural products. This survey reveals the important of herbs in drug discovery.²

ANTIOXIDANTS^{48,76}

Oxygen is essential for the survival of all living thing in the earth. Oxygen is being utilized in many physiological and metabolic processes of which about 5% gets reduced to free radicals. Free radicals are species with one or more unpaired electrons which reacts with electron acceptors such as molecular Oxygen to form reactive oxygen species (ROS). The various ROS are superoxide anion radicals (O_2^-). Hydrogen peroxide, hydroxyl radical and singlet O_2 . The short lived ROS reacts with non radicals and produce chain reactions.

Antioxidants are substances which delays oxidation process, inhibit the polymerization chain reaction, counter act the oxidative damage due to ROS and thereby improve the quality of life. They may provide protection against chronic diseases such as cancer, neurodegenerative diseases, inflammatory and cardiovascular diseases.

The antioxidant enzymes includes superoxide dismutase (SOD), peroxidase (P-ase), catalase (C-ase) and some natural antioxidants include GSH reduced Glutathione and phenolic compounds (tocopherol, flavonoid, phenolic acids).

Many plants contain substantial amount of antioxidants including vitamin C, vitamin E, carotenoids, flavanoids, tannins etc., and thus can be utilized to scavenge the excess free radicals from human body.

HEART FAILURE^{4,5,6,7}

Heart failure is a clinical syndrome that occurs in patients who because of an inherited or acquired abnormality of cardiac structure and or function, develop a constellation of clinical symptoms (dysnea and fatigue) and signs (edema and rales) that lead to frequent hospitalization a poor quality of life and a shortened life expectancy.

ETIOLOGY

- I) Depressed ejection fraction (<40%)
 - A) Coronary artery disease – myocardial infarction, myocardial ischemia.
 - B) Chronic pressure overload- hypertension, obstructive valvular disease
 - C) Chronic volume overload- regurgitant valvular disease, intracardiac shunting, extracardiac shunting.
 - D) Nonischemic dilated cardiomyopathy- familial genetic disorder, infiltrative disorders, toxic/drug induced damage, metabolic disorder, viral, chronic disease.
 - E) Disorder of rate and rhythm-chronic bradyarrhythmia, chronic tachyarrhythmia.
- II) Preserved ejection fraction (>40-50%)
 - A) Pathological hypertrophy - Primary (hypertrophic cardiomyopathies);Secondary (hypertension)
 - B) Restrictive cardiomyopathy - Infiltrative disorders (amyloidosis, sarcoidosis) ;Storage disease (hemochromatosis); Fibrosis - Endomyocardial disease

III) Pulmonary heart disease

Pulmonary vascular disorders

IV) High-output states

Metabolic disorders, Thyrotoxicosis, Nutritional disorders (beriberi),

Excessive blood flow requirement, Systemic arteriovenous shunting,

Chronic anemia

DIAGNOSIS

1. Routine laboratory testing
2. Electrocardiogram
3. Chest X-ray
4. Assessment of LV function
5. Biomarkers
6. Exercise testing

PHARMACOLOGICAL MANAGEMENT

CHRONIC HEART FAILURE

1. Diuretics
2. Angiotensin converting enzyme inhibitors
3. Angiotensin receptor blockers
4. β -receptor blockers
5. Anticoagulant and antiplatelet therapy

ACUTE HEART FAILURE

1. Vasodilators
2. Inotropic agents
3. Vasoconstriction

CARDIOMYOPATHY

Cardiomyopathy are a group of diseases that primarily affect the heart muscles and are unable to pump the blood throughout the body.

CLINICAL CLASSIFICATION OF CARDIOMYOTHS

1. Dilated cardiomyopathy

Left or right ventricular enlargement, impaired systolic function, congestive heart failure, arrhythmias, embolic.

2. Restrictive cardiomyopathy

Endomyocardial scarring or myocardial infiltration resulting in restriction of left and or right ventricular filling.

3. Hypertrophic cardiomyopathy

Disproportionate left ventricular hypertrophy, typically involving septum more than free wall, with or without an intraventricular systolic pressure gradient, usually of a non dilated left ventricular cavity.

CARDIOTOXICITY

Cardiotoxicity is a condition where the damage of heart muscles occur and the heart may not be able to pump the blood throughout the body. Severe cardiotoxicity leads to cardiomyopathy.

CARDIOTOXIC AGENT

A variety of pharmacologic agents may damage the myocardium acutely, producing a pattern of inflammation (myocarditis) or may even lead to chronic damage as seen in DCM. Certain drugs may produce ECG abnormalities alone while others may precipitate CHF and death. Some of the cardiotoxic agents include doxorubicin, daunorubicin, cyclophosphamide, phenothiazine, chloroquine, cocaine, trastuzumab etc.

Doxorubicin, an anthracyclic derivative is a powerful antineoplastic agent but severe cardiomyopathy and heart failure have been observed in doxorubicin treated cancer patients hence its use being limited. It causes lipid peroxidation of the myofibre membranes resulting in myofibre swelling, vacuolization, fatty changes, cytolysis systolic dysfunction and ventricular tachyarrhythmic occurs in a dose dependent manner.

CARDIOPROTECTIVE HERBS

Digitalis purpurea

Strophanthus kombe

Gymnema sylvestre

Terminalia arjuna

Euodia officinalis

RHEUMATOID ARTHRITIS^{8,9,10}

Rheumatoid arthritis is traditionally, a chronic inflammatory autoimmune disorder (where the body own immune system attacks itself deliberately). It is a systemic disorder and the systemic manifestations include homological, pulmonary, neurological and cardiovascular abnormalities. The joints most commonly affected were neck, shoulder, elbows, hip, knees and ankles.

EPIDEMIOLOGY

Rheumatoid arthritis affects almost 1% of the adults of the developed population in the world. Ten years after the onset of the disease, 60% of the patients showed significant disability and after 20 years mark, more than half of the patients showed severe disability or even dead. The highest mortality in rheumatoid arthritis population is directly attributed to excess cardiovascular disease. These patients suffer more myocardial infarction, cerebrovascular accidents and even heart failure than those without rheumatoid arthritis.

ETIOLOGY

- ❖ Etiology is not clearly known
- ❖ It occurs in immunogenetically predisposed individuals to the effect of microbial agents acting as trigger antigens.
- ❖ Due to the existence of infectious agents lack as mycoplasma, Epstein barr virus (EBR) cytomegalovirus (CMV) or rubella.

SIGNS AND SYMPTOMS

- ❖ Pain and swelling of joints in symmetrical fashion.
- ❖ Onset of disease begins with fatigue, weakness, morning stiffness, arthralgia, myalgia, redness and difficult movement.
- ❖ Rheumatoid nodules also seen close to the joints of neck, shoulder, elbow, hip, knees and ankles.

DIAGNOSIS

- ❖ Clinicians usually depend on the presence or absence of rheumatoid factor (RF) in the blood. But this diagnosis is not always correct.
- ❖ In advanced cases radiological abnormalities such as narrowing of joint space and ulnar deviation of fingers and radial deviation of wrist are seen in x-ray diagnosis.
- ❖ Laboratory findings include mild normocytic, normochromic anemia, elevated ESR, mild leucocytosis and hypergamma globulinemia.
- ❖ Anticyclic citrullinated peptide (CCP) antibody test is a recent serological diagnostic tool.

TREATMENT

- ❖ NSAIDs were used first to afford symptomatic relief from pain, swelling, morning stiffness, immobility, but do not arrest the disease.
- ❖ Disease modified anti arthritic drugs includes – gold, d-penicillamine, chloroquine, sulfasalazine, leflunomine, immunosuppressants. These are given NSAIDs have failed or when deformity and bony changes progressive rapidly.

HERBAL REMEDIES

- ❖ *Ficus benghlensis.*
- ❖ *Capsicum annum*
- ❖ *Menthe arvensis*
- ❖ *Alpinia officinarum*
- ❖ *Aconitum napellus*
- ❖ *Trachyspermum roxburghianum*

RHEUMATIC FEVER^{7,8,11}

Rheumatic fever is a systemic, post-streptococcal non-suppurative inflammatory disease, principally affecting the heart, joints, central nervous system, skin and subcutaneous tissues. The chronic stage of rheumatic fever involves all the layers of the heart (pancarditis) leading to rheumatic heart diseases (RHD).

EPIDERMIOLOGY

This disease appears most common in childrens between 5 to 15 years of age when the streptococcal infection is more frequent and intense. The incidence of disease has declined in the developed countries but still prevails in developing countries like India, Pakistan, parts of Africa and South America. About 75% of rheumatoid fever patients have arthritis and 2/3 of patients have carditis.

ETIOLOGY

Certain strains of beta haemolytic streptococcal cause pharyngitis and provoke the necessary antibodies for the causation of rheumatic fever.

STREPTOCOCCUS DISEASES

STREPTOCOCCUS PYROGENS

1. Pyrogenic infection

- ❖ Respiratory tract - acute tonsillitis or pharyngitis (sore throat), scarlet fever
- ❖ Skin infection - infection of wound, burns etc.
- ❖ Deep infection - bone and joint infection, septicaemia, abscess in internal organs.

2. Non-suppurative complications

Rheumatic fever, acute glomerulonephritis.

ENTEROCOCCUS FAECALIS

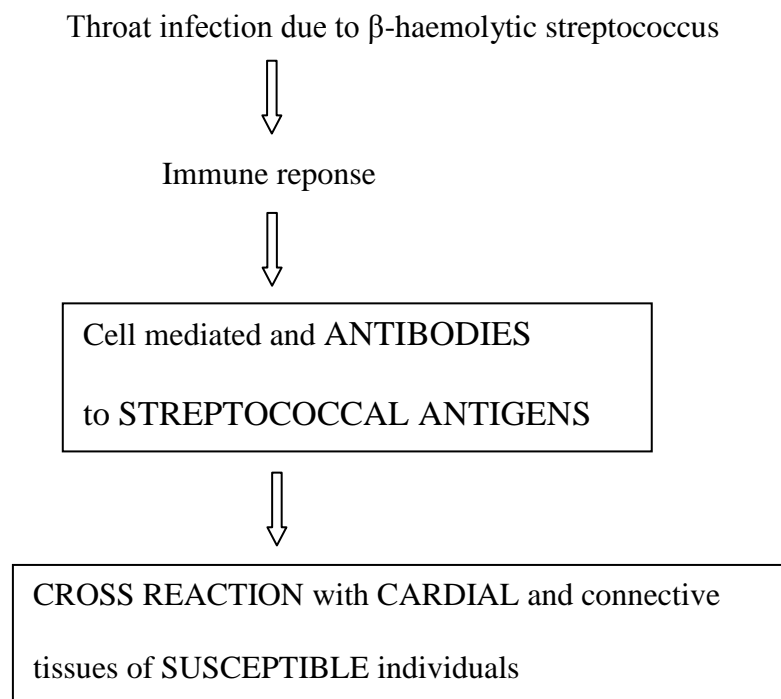
Endocarditis.

STREPTOCOCCI VIRIDANS

Endocarditis and dental caries.

MECHANISMS

Throat infection due to β -haemolytic streptococcus (Group A) – immune response – cell mediated and antibodies to streptococcal antigens – cross reaction with cardiac and connective tissues of susceptible individual.



When the immune system begins to attack the heart, it may attack any of the 3 layers (pancarditis) of the pericardium, myocardium and endocardium. The causative organism not only causes the cardiac manifestations it also leads to extra-cardiac manifestations like polyarthrititis, subcutaneous, nodules, erythema marginatum, rheumatic arthritis etc.

TREATMENT

Rheumatic fever can be stopped to be transformed to rheumatic heart diseases by using antibiotics against *Streptococcus pyrogens*. Penicillin is the drug of choice.

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CERTIFICATE

This is to certify that the dissertation entitled **“Pharmacognostical, Phytochemical including isolation of phenolic rich fraction, HPTLC analysis and Pharmacological studies on the leaves of *Xanthium strumarium (linn)*”** was done by **Miss. S. PADMA THANGA PARAMESWARI**, in Department of Pharmacognosy, Madurai Medical College, Madurai-20, in partial fulfilment of the requirement for the Degree of Master of Pharmacy in Pharmacognosy. This dissertation is forwarded to the Controller of Examination, The Tamilnadu Dr. M.G.R. Medical University, Chennai.

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Place:

Date:

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CHAPTER 2

LITERATURE SURVEY

ETHNOMEDICAL INFORMATION^{13, 14, 15}

1. In Ayurveda, it was stated that the plant possess anthelmintic, antipyretic, alexitaric, tonic, digestive activity and improves appetite, memory, voice and complexion. It was also used to cure leucoderma, poisonous bites of insects, epilepsy, salivation and fever.
2. The Native American tribes used this plant to relieve constipation, diarrhoea and vomiting
3. The Chinese used this plant for headache, ulcer, sinus problems.
4. The plant was considered to be useful in the treatment of long standing case of malaria.
5. An infusion of the plant has been used to treat rheumatism, kidney diseases and tuberculosis.
6. The fruit has been used as antibacterial, antifungal, antimalarial, antispasmodic, antitussive, cytotoxic, hypoglycaemic and stomachic
7. Historically the roots were being used in the treatment of scrofulous tumours, ulcers, boils and abscesses.
8. The juice of leaves and fruits were believed to be used for small pox and roots are used for cancer .
9. Decoction of the seeds were used for the treatment of bladder complaints.
10. The plant is considered as medicinal plant of West Bengal and wild edible plant of Meghalaya.

LEAVES

PHYTOCHEMISTRY

1. Boyer, Gregory L et.al (1986) isolated and identified 7'- hydroxy(-)-R-abscisic acid by mass spectroscopy and optical rotation dispersion as a metabolite of feeding (-)-R abscisic acid to *X. strumarium*.¹⁶
2. Bisht NPS et. al (1979) isolated strumasterol, oleic acid, 3,4 – dihydroxy cinnamic acid, β sitosterol- D-glucoside from the leaves of *X. strumarium*.¹⁷
3. Ahn. J. W. et.al (1996) isolated the cytotoxic compound (xanthatin, 8-epi-xantatine, 8-epi-tomentosin) from the leaves of *X. Strumarium*.¹⁸

PHARMACOLOGY

1. Reddy UM et.al (1981) studied the antifungal activity against (*Drechslera rostrata*, *Curvularia lunata* and *Furarium oxysporum*) and antibacterial activity against (*E.coli*, *Proteus vulgaris*, *Klebsiella species*, *Pseudomonas fluorescens*, *sarcina lutea* and *Bacillus megaterium*) leaf extract of *X. strumarium*.¹⁹
2. Misra SB et.al (1982) studied the antifungal activity against *Ustilago tritici*, *U. Hordei*.²⁰
3. Kishore N et.al (1983) showed that ethanolic extract of *X. strumarium* completely inhibited the mycelial growth of *F. Momiliforme*.²¹
4. Nandal SN et.al (1984) screened *X. strumarium* for nematocidal activity against *Meloidogyne javanica*.²²
5. Renu et. el (1984) studied the fungal toxicity of leaf extract of *X. strumarium* against *Rhizoctonia solani kushm*.²³

6. Dongre TK et.al (1986) studied that leaf extract of *X. strumarium* decreases the egg laying of *Eariasvitella F.*²⁴
7. Akarte A.S. et.al (2010) evaluated the antinociceptive effect of leaf extract of *X. strumarium.*²⁵
8. Kim YS et.al (2003) isolated two xanthanolide sesquiterpene lactones (8-epi-xanthatin and 8-epi-xanthatin epoxide) from the leaves of *X. strumarium* and demonstrated the inhibition of the proliferation of cultured human tumour cells.²⁶
9. Talakal et.al (1995) screened for both invitro and invivo antitrypanosomal activity of leaf extract of *X. Strumarium*²⁷
10. Torres, J. Molina et.al (1991) studied the diurnal changes in the incorporation of (U-14 C) tyrosine into tocopherols and plastoquinone in leaves of *X. Strumarium.*²⁹
11. Scherer et.al (2009) studied the antioxidant activity on the methanolic extract by DPPH and nitric oxide scavenging methods..³⁰

PHARMACOGNOSY

1. Keese RJ et.al (2006) studied the effect of monosodium methanarsonate on cuticle wax content of *X. strumarium.*³¹

FRUITS

PHYTOCHEMISTRY:

1. Ma Y.T. et.al (1999) isolated new thiazinedione (7-hydroxymethyl-8,8-dimethyl 1-4, 8-dihydrobenzo {1-4} thiazine-3,5- dione) and phenolic

compounds (caffeic acid, potassium 3-O-caffeoyl quinate, 1,5- di-O-caffeoyl quinic acid; 1,3,5- tri –O- caffeoyl quinic acid.³²

2. Quin .L. et.al (2007) isolated new thiazinedione, xanthiazone, chlorogenic acid, ferulic acid, formononetin and ononin from the fruits of *X. strumarium*.³³
3. Agata Isao et.al (1993) isolated 1,3,5-tri-O-caffeoylquinic acid and 3,5-di-O-caffeoylquinic acid from the fruits of *X. strumarium*. .³⁴

PHARMACOLOGY

1. Lee B.H et.al (2009) isolated 3,4-dihydroxy benzaldehyde and studied the apoptotic cell death through inhibition of protein kinase CK II activity on fruits of *X. strumarium*. .³⁵
2. Hsu FL et.al (2000) isolated the active principle, caffeic acid and studied the antihyperglycemic effect on the fruits of *X. strumarium*. .³⁶
3. Hong SH et.al (2003) studied the inhibitory effect on mass cell mediated allergy reactions on fruits of *X. strumarium*. .³⁷

PHARMACOGNOSY

1. Hamada T et.al (1989) reported the pharmacognostical studies on fruits of *X. Strumarium* .³⁸

SEEDS

PHYTOCHEMISTRY

1. Cole RJ et.al (1981) isolated and redefined the toxic agent (carboxyattractyloside) from the seeds of *X. strumarium*. .³⁹
2. UI' Chenko, N.T. et. al (2001) analysed neutral lipids,ordinary and hydroxylated fatty acids from the seeds of *X. strumarium*. .⁴⁰

PHARMACOLOGY

1. Lee C.L. et. al (2009) isolated xanthialdehyde and (-)- xanthienopyran inhibitors of superoxide anion generation by activated neutrophils from the seeds of *X. strumarium*. .⁴¹
2. Bark KM et.al (2010) evaluated the phytotoxic potential of seed oil of *X. strumarium*. .⁴²

AERIAL PART

PHYTOCHEMISTRY

1. Bohlmann F. et.al (1982) isolated 2-epi-xanthumin and 8-epi-xanthatin-1 β , 5 β -epoxide from the aerial part of *Xanthium indicum*. .⁴³
2. Ahmed A.A. et .al (2000) isolated xanthanolide diol derivate (11 alpha 13-dihydroxy xanthiatin and dimeric xanthanolide sesquiterpene lactone, pungiolide C and identified from the aerial parts of *X. strumarium*. .⁴⁴
3. Malik et.al (1982) isolated sesquiterpene lactone (2-hydroxytomentos 1 [beta] 5[beta]-epoxide from the aerial part of *X. strumarium*. .⁴⁵

PHARMACOLOGY

1. Joshi S.P et al (1997) studied the antimalarial activity from the acetone extract of aerial parts of *X. strumarium*. .⁴⁶
2. Badam L. et. al (1988) studied the invitro antimalarial activity on ethanolic extract of aerial part of *X. strumarium*. .⁴⁷

CHAPTER 3

AIM AND SCOPE OF THE PRESENT STUDY

The ethnomedical information of the plant reveals that the leaves of *Xanthium strumarium* linn. were used as antirheumatic, antisyphilitic, diuretic, antitubercular, antimalarial, laxative and sedative . According to siddha system of medicine, leaves of this plant possesses cardioprotective property. The fruits of this plant were found to possess antibacterial and antifungal activities.

The phytochemical studies on the leaves have been reported for the presence of flavanoids, tannins, alkaloids, sterols and carbohydrates. Some phytochemical studies have been documented for the presence of phenolic compounds in this plant.

The cardioprotective and anti-arthritis activities have not yet been reported so far on the leaves of *Xanthium strumarium*.(X.S)

Hence the present work has been designed in such a way as to carry out the following studies on the leaves *Xanthium strumarium* linn.

1. Pharmacognostical studies on the leaves.
2. Preliminary phytochemical screening on the extracts of X.S.
3. Isolation of phenolic rich fractions from ethanolic extract of *X.strumarium*
4. Estimation of total phenols, total tannins and total flavanoids.
5. Phytochemical evaluation of both extract and phenolic rich fraction by TLC and HPTLC studies.

6. Screening of the ethanolic extract and phenolic rich fraction of the leaves for following pharmacological activities.

- Invitro antioxidants activity
 - DPPH radical scavenging activity
 - Nitric oxide scavenging activity
 - Phosphomolybdenum method
- Cardioprotective effect of leaf extract of *Xanthium strumarium* on Doxorubicin induced cardiotoxicity in rats.
- Invitro anti-arthritic activity
- Antibacterial activity against *Streptococcus pyogenes* causative organism for rheumatic fever and *Streptococci viridians*, *Enterococcus faecalis* causative organism for endocarditis.

CHAPTER - 4

PHARMACOGNOSTICAL STUDIES

SECTION – A

Plant profile^{49,50}

BIOLOGICAL SOURCE : *Xanthium Strumarium* Linn.

FAMILY : Asteraceae (Compositae)

Xanthium Strumarium is a dicotyledonous, monoecious herb. It is a course unarmed annual plant upto 1.5 m height and commonly found as weed.

SYSTEMATIC POSITION:

Kingdom : Plantae

Subkingdom : Tracheobionta

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Asteridea

Order : Asterales

Family : Asteraceae

Genies : *Xanthium*

Species : *strumarium*

SYNONYM:

Xanthium indicum Koen.

VERNACULAR NAMES:

English : Burweed, Clotbur, Cocklebur.

Hindi : Borkra, Gokhru, Chota – gokhru, adhasisi.

Kannada : Maruluummatti

Malayalam : Buah anjang

Sanskrit : Arishta, Shankine.

Tamil : Maruloomatham

Telugu : Marulamathangi

Bengali : Banokra, Chota – dhatura.

Marathi : Dumundi, dutundi.

Gujaratti : Gadriyun

GEOGRAPHICAL DISTRIBUTION:

Cocklebur is probably a native of America. It has become naturalized in India and has spread rapidly in several parts of Madhya Pradesh, Maharashtra, at an altitude of 1500m in Himalayas. Cocklebur was cultivated as a leafy vegetable in china.

HABIT AND HABITAT:

Xanthium Strumarium is an annual herb of about 1.5m tall with short, stout, slightly branched stem with short hairs over it. It is distributed in most plain districts on waste ground and road sides. *Xanthium strumarium* has wide distribution because of the ease with which its inflorescence is carried about by the feet of the animals and also in their wool

MORPHOLOGICAL STUDIES ON XANTHIUM STRUMARIUM

Plant description ^{51,52,53}

Leaves

Numerous dark green and alternative leaves of about 5-7cm long and as broad as long. Triangular – cordate or suborbicular in shape often three lobed. Irregular toothed margin and cuneate apex. Rough with appressed hairs on both sides of the leaves. Petiole is 2.5 – 7cm long and hairy.

Flowers

Flower heads are numerous with terminal and axillary racemes. Barren heads are numerous and crowded at the top of the stem while fertile heads are fewer and axillary. There are few involucre bracts and many inside covered with hooked bristles. There are two flowers with no pappus and corolla.

Fruits

The fruits are large celled with strong hooks. Achenes are obvoid and closed in the hardened involucre . Fruits are pubescent, thickly clothed with hooked prickles and about 1.6cm long with two erect mucronate beaks.

SECTION - B

MICROSCOPICAL STUDIES ON THE LEAVES OF

XANTHIUM STRUMARIUM ^{54,55,56}

COLLECTION OF SPECIMEN

The plant specimens for the proposed were collected from road side of Madurai. Care was taken to select healthy plants and normal organs. The required samples of leaf were cut and removed from the plant and fixed in FAA (formalin-5ml+acetic acid-5ml+70%ethyl alcohol-90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the schedule given by Sauss, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 c) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

SECTIONING

The paraffin embedded specimen was sectioned with the help of rotary microtome. The thickness of the sections was 10-12µm, dewaxing of the sections was by customary procedure (Johansen, 1940). The sections were stained with toluidine blue as per the method published by O'BRIEN et al. (1964). Since toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with saffranin and fast-green and iodine (for starch)

For studying the stomata morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass,1940) were prepared. Glycerin mounted temporary preparations were made for macerated/ cleared material. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell component were studied and measured.

PHOTOMICROGRAPHS

Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observation bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnification of the figures were indicated by the scalebars.

1. Leaf:

The leaf consists of very thick midrib and thin lamina (Fig: 1.1). The midrib is broadly hump shaped on the adaxial side and broadly semicircular on the abaxial side. The midrib is 1.35mm thick, the adaxial hump is 300mm in height and 480mm wide. The abaxial part is 1.2mm wide (Fig; 1.2).

The midrib consists of uniformly thin epidermal layer of squanesh small cells. Inner to the epidermis are three or four layers of collenchyma cells in the adaxial hump as well as abaxial end. The ground tissue is parenchymatous, the cells being large, circular to angular and compact. Distributed in the ground

tissue are small circular secretory canals, with a ring of small epithelial cells (Fig : 1.2)

The vascular system of the midrib is multistranded. There are large discrete collateral vascular bundles about five bundles are arranged in a shallow arc along the abaxial part and one or two bundles located in adaxial part. The vascular bundles have 3-5 parallel lines of circular thick walled xylem elements and thick, semicircular cap of phloem elements (Fig : 1.2).

Lamina (Fig: 2.1-3.3, 1-3)

The lamina is thin, dorsiventral and amphistomatic (stomata present on both sides of the lamina). The adaxial surface even while abaxial surface has shallow pits in which epidermal trichomes are located (Fig : 2.1,2.3). The adaxial epidermis and abaxial epidermis comprises of narrow cylindrical. The mesophyll tissue is differentiated into two adaxial layers of narrow cylindrical, less compact palisade cells and abaxial zone of 7-10 layers of small lobed spongy parenchyma cells which form air-chambers (Fig : 2.1, 2.3). The lamina is 250µm thick. The lateral veins do not project much beyond the level of the lamina. The vascular strand of the lateral vein is small and collateral. It consists of adaxial xylem and abaxial phloem, the vascular strands is enclosed by a circular layer of parenchymatous bundle sheath with adaxial and abaxial extensions (Fig : 2.3).

Epidermal Trichomes:

Nonglandular and glandular trichomes are seen on both the abaxial and adaxial sides of the lamina (Fig : 2.1-3, 3.1). The nonglandular trichomes are more predominated than the glandular type. The nonglandular trichomes are 2-5 celled, uniseriate,

unbranched and thin walled. The basal cell of the trichome is widest and the cells above in the trichome are gradually narrow ending in a pointed end (Fig : 3.2). The trichomes are 140µm in height, the basal cell is 60µm wide and the terminal cell is 10µm wide.

Glandular trichomes are not much prominent. They are very small, and delicate, dumbshell shaped and 2 celled. They are situated in shallow pits of the abaxial epidermis (Fig : 4.1, 2). The stalk of the gland is very short. The gland is 50 µm in horizontal plane (Fig ; 6.2).

Stomata:

In surface of the paradermal sections, epidermal cells appear small, polygonal with slightly wavy anticlinal walls (Fig : 4.1, 4.2, 5.1). The stomata are dense and random in distribution. They are actinocytic type in which a stoma is surrounded by five or more radiating triangular subsidiary cells (Fig : 4.12). The guard cells are elliptical with wide stomatal pore. The stoma is 20x25 µm in size.

Venation (Fig : 5.12, 6.1):

The minor veins that from the areoles are thin with single row of vascular elements. The vascular strand is ensheathed throughout its course by dilated, hyaline parenchymatous bundle sheath (Fig : 5.2). The vein-islets are well defined with distinct boundaries. The islets are narrow, tetragonal to hexagonal in outline. The vein-terminations are lacking in most of the islets. When present the terminations are short, and less prominent.

Petiole:

The petiole was studied along the three regions, viz; basal part (Fig : 7.1.2), middle part (Fig : 8.2) and terminal part (Fig : 9.1,2,3). The petiole is circular with shallow adaxial depression in the basal and middle parts. Along the terminal (distal) part it is 3 lobed with wide shallow adaxial concavity. The basal part is 3.1mm thick; the middle part is 4mm thick. The terminal part is less than 4mm thick. In all three regions the petiole has thin discrete epidermal layer followed by four or five layers of collenchymas cells and the rest of the ground tissue consists of large thin walled compact parenchyma cells. Secretory canals with epithelial cells are scattered in the ground tissue.

The vascular system exhibits much variation in the three regions of the petiole. In the basal part, there is a large central abaxial vascular strand and an adaxial row of comparatively smaller three bundles. These are also much smaller less prominent vascular strands placed in between the major bundles (Fig : 7.1,2).

In the middle region are three larger and four smaller bundles arranged in an arc (Fig : 8.1). In the terminal part, the vascular strands are multiplied forming a lower arc of 6 strands and upper arc of 8 strands (Fig : 9.1). All bundles are collateral with many parallel rows of circular wide xylem elements, a thin band of phloem elements and thick hemispherical bundle cap sclerenchyma. Except variation in number and size of the vascular strands, the basic type and stomata are similar.

SECTION – C

POWDER ANALYSIS

In the leaf powder are seen small fragments of lamina, epidermal peeling and epidermal trichomes.

- Fragmentary leaf bits exhibit the venation pattern. The venation is densely reticulate. The veins are thin with prominent parenchymatous sheath all along the veins. The vein-islets are small, polyhedral and well demarcated by distinct vein boundaries (10.1,2). These vein terminations are mostly simple, short, branched or more commonly unbranched.
- Epidermal trichomes of nonglandular type are common in this powder. They are multicellular walled. The trichome is wider at the base and becomes abruptly sharper at the tip. No inclusions are evident in the cells.

SECTION – D

QUANTITATIVE MICROSCOPICAL STUDIES ^{57,58}

DETERMINATION OF LEAF CONSTANTS:

The vein islet number, vein termination number, stomatal number and stomatal index were determined on fresh leaves by using standard procedures.

A. Vein islet number and vein termination number:

The term vein islet is used to denote the minute area of photosynthetic tissue encircled by the ultimate division of the conducting strands. The number of vein islets per square mm area is called vein- islet number.

Vein termination number may be defined as the number of vein terminations present in one square mm area of the photosynthetic tissue.

Determination of vein islet number and vein termination number:

Small pieces of leaves were cut on the lamina between midrib and the margin, cleared in chloral hydrate and mounted on a slide.

Camera Lucida and drawing board were arranged. With the help of a stage micrometer, camera Lucida and microscope, 1mm square was drawn on the paper. Then the stage micrometer was replaced by the sample slides and the veins were traced over the square. The vein islets and vein terminations were counted in the square. Ten such readings were taken and the average was calculated and the results were presented in

TABLE-1

A. Stomatal Number:

The average number of stomata per square mm area of epidermis of the leaf is called stomatal number.

Determination of Stomatal Number

Small pieces of upper and lower epidermal peelings of the leaves were mounted on a slide. Camera Lucida and stage micrometer were used to draw 1 mm square was on a paper. The stage micrometer was replaced by the preparation slide. Then the preparation were observed under microscope and the stomata were marked in that unit area. Number of stomata present in those unit area were calculated. Ten such readings were taken and the average of stomatal number was calculated and presented in the TABLE- for both upper and lower epidermis.

B. Stomatal Index:

Definition:

It is the percentage which the numbers of stomata form to the total number of epidermal cells, each stoma beings counted as one cell.

Stomatal index

$$S.I = \frac{S}{E+S} \times 100$$

Where, S=number of stomata per unit area

E= number of epidermal cells in the same unit area

Determination of Stomatal Index:

The procedure adopted in the determination of stomatal number was followed and the preparation was observed under high power. The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the above formula and was given in **TABLE-1**

Table 1: Quantitative microscopical parameters of the leaf of *Xanthium strumarium*.

S. No.	Parameters*	Values obtained*
1	Stomatal number in upper epidermis	103.26 \pm 0.12
2.	Stomatal number in lower epidermis	227.43 \pm 0.32
3.	Stomatal index in upper epidermis	17.63 \pm 0.34
4.	Stomatal index in lower epidermis	26.54 \pm 0.63
5.	Vein islet number	13.49 \pm 0.13
6	Vein termination number	2.63 \pm 0.24

***Mean of 6 readings \pm SEM**

SECTION – E

PHYSICAL PARAMETERS ^{57,59,60,61}

ASH VALUES:

The ash values were determined by using air dried powdered leaves as per the official method.

(A) Total ash:

2 grams of the crude leaf powder were accurately weighed in a tarred nickel crucible. The ground drug was scattered in a fine even layer on the bottom of the crucible and incinerated by gradually increasing the heat not exceeding 450° c [dull red heat] until free form carbon. Then it was cooled and weighed for constant weight. The percentage of ash with reference to the air dried drug were calculated.

(B) Acid insoluble ash:

The ash obtained in (A) were boiled for 5 minutes with 25ml of 2M hydrochloric acid. The insoluble matter was collected in a tarred sintered glass crucible. The residue was washed with hot water, ignited to constant weight, cooled in a desiccators and weighed. The percentage of acid insoluble ash with reference to the air dried drug were calculated.

(C) Water soluble ash:

The ash obtained in (A) was boiled with 25ml of distilled water. The insoluble matter was collected in a Gooch crucible, washed with hot water ignited to a constant weight. Cooled in a desiccators and weighed. The weight of the insoluble matter was

subtracted from the weight of the total ash. The difference gave the weight of the water soluble ash. It was calculated with reference to the air-dried powder. The results were represented in **TABLE-2**

C. Loss on drying:

Loss on drying was determined by the method described by Wallis. 2 grams of the powdered crude drug was accurately weighted in a tarred dish and dried in an oven at 100°-105°C. It was cooled in a desiccator and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken and presented in **TABLE -.2**

Table 2: Analytical parameters of *X.strumarium*

S. No	Parameters*	Values* expressed as %
1	Ash values	
	Total ash	11.42 ± 1.34
	Water insoluble	6.57 ± 0.95
	Water soluble ash	4.85 ± 0.74
	Acid insoluble ash	2.49 ± 0.62
2	Loss on drying	2.14 ± 0.31

*** mean of three readings**

Determination of Extractive values:

I) Petroleum ether soluble extractive value

5 gm of the air –dried drug, coarsely powdered, was macerated in 100ml of petroleum ether in a closed flask for 24 hours, shaking frequently during 6 hrs and allowed stand for 18 hrs, filtered rapidly, taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105° c, to constant weight. The percentage of the petroleum ether soluble extractive with references to the air - dried drug was calculated as presented in **TABLE-3**

II . Alcohol (Ethanol) and 75% ethanol soluble extractive:

5 gm of the air –dried drug, coarsely powdered, was macerated in 100ml of ethanol in a closed flask for 24 hours, shaking frequently during 6 hrs and allowed stand for 18 hrs, filtered rapidly, taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105° c, to constant weight. The percentage of alcohol soluble extractive with references to the air - dried drug was calculated as presented in **TABLE-3**

III). Water-Soluble Extractive:

5 gm of the air dried drug coarsely powdered, was macerated in 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during 6 hours, and allowed to stand for 18 hours. Filtered rapidly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred-flat-bottomed shallow dish and

dried at 105° C to constant weight. The percentage of water – soluble extractive with reference to the air-dried drug was calculated and presented in **TABLE -3**

IV). Ether soluble Extractive:

The above mentioned procedure for the determination of petroleum ether soluble extractive was followed for the determination of ether soluble extractive also.

V). Determination of Chloroform, Acetone, methanol and hexane soluble extractives:

The procedure followed for the determination of alcohol soluble extractive value was adopted for the determination of Chloroform soluble extractive, acetone soluble extractive, methanol soluble extractive and hexane soluble extractive. Instead of alcohol, respective solvents were used for the determination of their extractive values.

The percentage of chloroform, acetone, methanol and hexane soluble extractives were calculated and presented in **TABLE-3**

Table 3: Extractive values of *X.strumarium*

S. No	Parameters*	Values* expressed as %
	Extractive values	
1	Petroleum ether extract	0.4765
2	Ether extract	3.517
3	Hexane	3.318
4	Chloroform	4.015
5	Acetone	1.559
6	Methanol	3.289
7	Ethanol	2.335
8	75% Ethanol	6.437
9	Aqueous	5.218

Power analysis

The behaviour of the *X.strumarium* powder with various chemical reagents is tabulated in **table 4** and that of fluorescence analysis of the powder and the extracts of *X.strumarium* are presented in **tables 5 & 6**. The powder showed the presence of phytosterols, tannins, proteins, flavanoids, phenolic compounds.

Table 4: Behavior of the *X.strumarium* powder with various chemical reagents

Powder + Reagents	Colour / Precipitate	Presence of active principle
Picric acid	Yellow precipitate	Protein present
Conc. sulfuric acid	Reddish brown color	Phyto sterols present
Lieberman Burchard reagent	Reddish brown color	Phyto sterols present
Aqueous ferric chloride	Greenish black color	Tannins present
Iodine solution	No blue color	Absence of starch
Mayer's reagent	Cream color	Alkaloids present
Spot test	No stain	Fixed oils absent
Sulfosalicylic acid	White precipitate	Protein present
Aq. Sodium hydroxide	Yellow color	Flavanoids present
Mg – HCl	Magenta color	Flavanoids present
Aq. Lead acetate	White precipitate	Presence of tannins

Note :- Colour reactions are viewed under natural light by naked eye

Table 5: Fluorescence Analysis of powder of *X.strumarium*

Powder +reagent	Day light	UV light (254 nm)	UV light (366 nm)
Drug powder	Green	Green	Brown
Drug powder +aqueous 1M sodium hydroxide	Greenish yellow	Green	Brown
Drug powder + alcoholic 1M sodium hydroxide	Green	Green	Brown
Drug powder + iodine	Red	Green	Brown
Drug powder + 10% potassium hydroxide	Yellowish green	Green	Brown
Drug powder + 1M hydrochloric acid	Green	Green	Brown
Drug powder + glacial acetic acid	Green	Green	Brown
Drug powder + 50% sulphuric acid	Green	Green	Brown
Drug powder + 50% nitric acid	Green	Dark green	Brown
Drug powder + 50% hydrochloric acid	Green	Green	Brown

Table 6: Fluorescence Analysis of extracts of *X.strumarium*

Extracts	Consistency	Colour in Day Light	Colour under UV Lamp	
			366nm	254nm
Petroleum extract	Semisolid	Yellowish green	Reddish orange	Green
Ether extract	Semisolid	Greenish yellow	Brown	Green
Chloroform extract	Semisolid	Greenish brown	Orange	Green
Hexane extract	Semisolid	Yellowish green	Orange	Green
Ethanol extract	Semisolid	Green	Orange	Green
Methanol extract	Semisolid	Green	Orange	Green
Aqueous extract	Semisolid	Greenish brown	Green	Greenish brown

CHAPTER-5

SECTION-A

PHYTOCHEMICAL STUDIES

COLLECTION AND AUTHENTICATION

The leaves of *Xanthium Strumarium* were collected in and around Madurai and authenticated by taxonomist. The leaves were washed thoroughly and dried in shade. The shade dried leaves were powdered and used for further studies.

ORGANOLEPTIC EVALUATION

- Nature - Coarse Powder
- Colour - Green
- Odour - no odour
- Taste - acrid

PRELIMINARY PHYTOCHEMICAL SCREENING ^{62,63,64,65}

1. Test for sterols

The powdered leaf was first extracted with petroleum ether and evaporated to a residue. Then the residue was dissolved in chloroform and tested for sterols.

a.Salkowski's Test

A few drops of concentrated sulphuric acid was added to the above solution, shaken well and set aside. The lower chloroform layer of the solution turned red in color indicating the presence of sterols.

b. Liebermann – Burchard's Test

To the chloroform solution a few drops of acetic anhydride and 1 ml of concentrated Sulphuric acid were added through the sides of the test tube and set aside

for a while. At the junction of two layers a brown ring was formed. The upper layer turned green indicating the presence of sterols.

2. Test for Terpenoids

A little of the powdered leaf was extracted with chloroform and filtered. The filtrate was warmed gently with tin and thionylchloride. Pink color solution appeared which indicated the presence of terpenoids.

3. Test for carbohydrates

a. Molisch's Test:

The aqueous extract of the powdered leaf when treated with alcoholic solution of α -naphthol in the presence of sulphuric acid gave purple color indicating the presence of carbohydrates.

b. Fehling's Test:

The aqueous extract of the powdered leaf was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. Red precipitate was obtained indicating the presence of free reducing sugars.

4. Test for Flavonoids

a. Magnesium turning- con HCl test:

A little of the powdered drug was heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added. Boiled for five minutes. Red colour was obtained indicating the presence of flavonoids.

b. Alkali Test

To the small quantity of test solution 10% aqueous sodium hydroxide Solution was added. Yellow orange color was produced indicating the presence of flavonols.

C. Acid Test

To the small quantity of test solution, few drops of concentrated Sulphuric acid was added. Yellow orange color obtained indicates the presence of flavonols.

7. Test for Proteins

a. Millon's Test

A small quantity of aciduous – alcoholic extract of the powdered drug was heated with Millon's reagent. White precipitate turned red on heating. Indicated the presence of proteins.

b. Biuret Test

To one portion of aciduous – alcoholic extract of the powdered drug one ml of 10% sodium hydroxide solution was added, followed by this one drop of dilute copper sulphate solution was added. Violet color was obtained indicating the presence of proteins.

8. Test for Alkaloids

a. About 2gm of the powdered material was mixed with 1gm of calcium hydroxide and 5ml of water into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. To this 200ml of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was evaporated. To this 5ml of dilute hydrochloric acid was added followed by 2ml of each of the following reagents.

- | | | |
|--------------------------|---|---------------------------|
| a) Mayer's Reagent | — | cream precipitate |
| b) Dragendorff's Reagent | — | orange brown precipitate |
| c) Hager's Reagent | — | Yellow precipitate |
| d) Wagner's Reagent | — | Reddish brown precipitate |

b. Test for purine group (Murexide test)

The residue obtained after the evaporation of chloroform as described in (a) was treated with 1ml of hydrochloric acid in a porcelain dish and 0.1gm of Potassium chlorate was added and evaporated to dryness on water bath. Then the residue was exposed to the vapour of dilute ammonia solution. No purple Color was obtained indicating the absence of purine group of alkaloids.

Test for Glycosides

Borntrager's Test

The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate, benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly.

No color reaction observed in ammoniacal layer showing the absence of Anthraquinone glycosides.

Modified Borntrager's Test

About 0.1g of the powdered drug was boiled for 2minutes with dilute hydrochloric acid and few drops of ferric chloride solution, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the Benzene extract.

No pink color was observed in ammoniacal layer showing the absence of anthraquinone glycosides.

Test for Cardiac Glycosides(for deoxysugar)

Keller Kiliani Test

About 1g of the powdered leaf was boiled with 10ml of 70% alcohol for 2 minutes, cooled and filtered. To the filtrate 10ml of water and 5 drops of solution of

leadsubacetate were added and filtered, evaporated to dryness. The residue was dissolved in 3 ml of glacial acetic acid. To this 2 drops of ferric chloride solution was added. Then 3 ml of concentrated sulphuric acid was added to the sides of the test tube carefully and observed.

No reddish brown layer was observed indicating the absence of deoxysugars of cardiac glycoside

Test for Cyanogenetic Glycosides

Small quantity of the powder was placed in a stoppered conical flask with just sufficient water, to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place.

No brick red color was produced on the paper indicating the absence of Cyanogenetic glycosides.

Test for saponins

About 0.5g of the powdered drug was boiled gently for 2 minutes with 20ml of water and filtered while hot and allowed to cool. 5 ml of the filtrate was then diluted with water and shaken vigorously. Frothing was produced indicating the presence of saponins.

Test for Tannins

A small quantity of the powdered drug was extracted with water. To the aqueous extract few drops of ferric chloride solution was added.

Bluish black color was produced indicating the presence of tannins.

Test for the presence of Volatile oil

Weighted quantity (250 gm) of fresh leaves were extracted the subjected to hydrodistillation using volatile oil estimation apparatus (BP 1980).

volatile oil was obtained indicating the presence of volatile oil

**TABLE: 4 PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE
LEAF POWDER OF *XANTHIUM STRUMARIUM***

S.NO	TEST	RESULTS
1.	TEST FOR STEROLS	
	a. Salkowski's test	+
	b. Libermann- burchard's test	+
2.	TEST FOR CARBOHYDRATES	
	a. Molisch's test	+
	b. Fehling's test	+
	c. Benedict's test	+
3.	TEST FOR PROTEINS	
	a. Millon's test	+
	b. Biuret test	+
4.	TEST FOR ALKALOIDS	
	a. Mayer's reagent	+
	b. Dragendroff's reagent	+
	c. Hager's reagent	+
	d. Wagner's reagent	+
5.	TEST FOR GLYCOSIDES	
	a. Anthraquinone glycosides	
	i) Borntrager's test	-
	ii) Modified Borntrager's test	-
	b. Cardiac glycosides	

	i) Keller Killiani test	-
	c. Cyanogenetic glycosides	-
6.	TEST FOR SAPONINS	+
7.	TEST FOR TANNINS	
	FeCl ₃ test	+
8.	TEST FOR FLAVONIDS	
	a. Shinoda test	+
	b. Alkali test	+
	c. Acid test	+
9.	TEST FOR TERPENIDS	+
10.	TEST FOR VOLATILE OILS	+

(+) indicates positive reaction

(-) indicates negative reaction

TABLE-5

**PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE VARIOUS
EXTRACTS OF LEAF POWDER OF *XANTHIUM STRUMARIUM***

Tests	Pet.ether Extract	Ether Extract	Hexane Extract	Chloroform Extract	Acetone Extract	Methanol Extract	Ethanol Extract	Ethanol extract (75%)	Aqueou s extract
I sterols									
Salkowski's test	+	+	+	+	+	-	-	-	-
Libermann - burchard's test	+	+	+	+	+	-	-	-	-
II. Test for Carbohydrates									
Molisch's test	-	-	-	-	-	+	+	+	+
Fehling's test	-	-	-	-	-	+	+	+	+
Benedict's test	-	-	-	-	-	+	+	+	+
III. Test for Proteins									
Millon's test	-	-	-	-	-	+	+	+	+
Biuret test	-		-	-	-	+	+	+	+

IV. Test for Alkaloids									
Mayer's reagent	-	-	-	-	-	+	+	+	+
Dragendrof f's reagent	-	-	-	-	-	+	+	+	+
Hager's reagent	-	-	-	-	-	+	+	+	+
Wagner's reagent	-	-	-	-	-	+	+	+	+
V. Test for Glycosides									
Anthraquinone glycosides	-		-	-	-	-	-	-	-
i) Borntrager's test	-		-	-	-	-	-	-	-
ii) Modified Borntrager's test	-		-	-	-	-	-	-	-
Cardiac glycosides									
i) Keller Killiani test	-	-	-	-	-	-	-	-	-

Cyanogenic glycosides	-	-	-	-	-	-	-	-	-
VI. Test for Saponins	-	-	-	-	-	+	+	+	+
VII. Test for Tannins									
FeCl ₃ test	-	-	-	-	+	+	+	+	+
VIII. Test for Flavonoids									
Shinoda test	-	-	-	-	+	+	+	+	+
Alkali test	-	-	-	-	-	+	+	+	+
Acid test	-	-	-	-	-	+	+	+	+
IX. Test for Terpenoid	+	+	+	+	+	+	+	+	-

(+) indicates positive reaction

(-) indicates negative reaction

SECTION-B

PREPARATION OF EXTRACT

About 1kg of the dried powdered leaf of *Xanthium Strumarium* was defatted with 2.5 litres petroleum ether (60-80⁰ C) by maceration. The solvent was removed by filtration and the marc is dried. To the dried marc 2.5 litres of 75% ethanol was added and the extraction was performed by triple maceration. It was then filtered and the combined filtrate was evaporated to a cohesive mars using rota vapour.

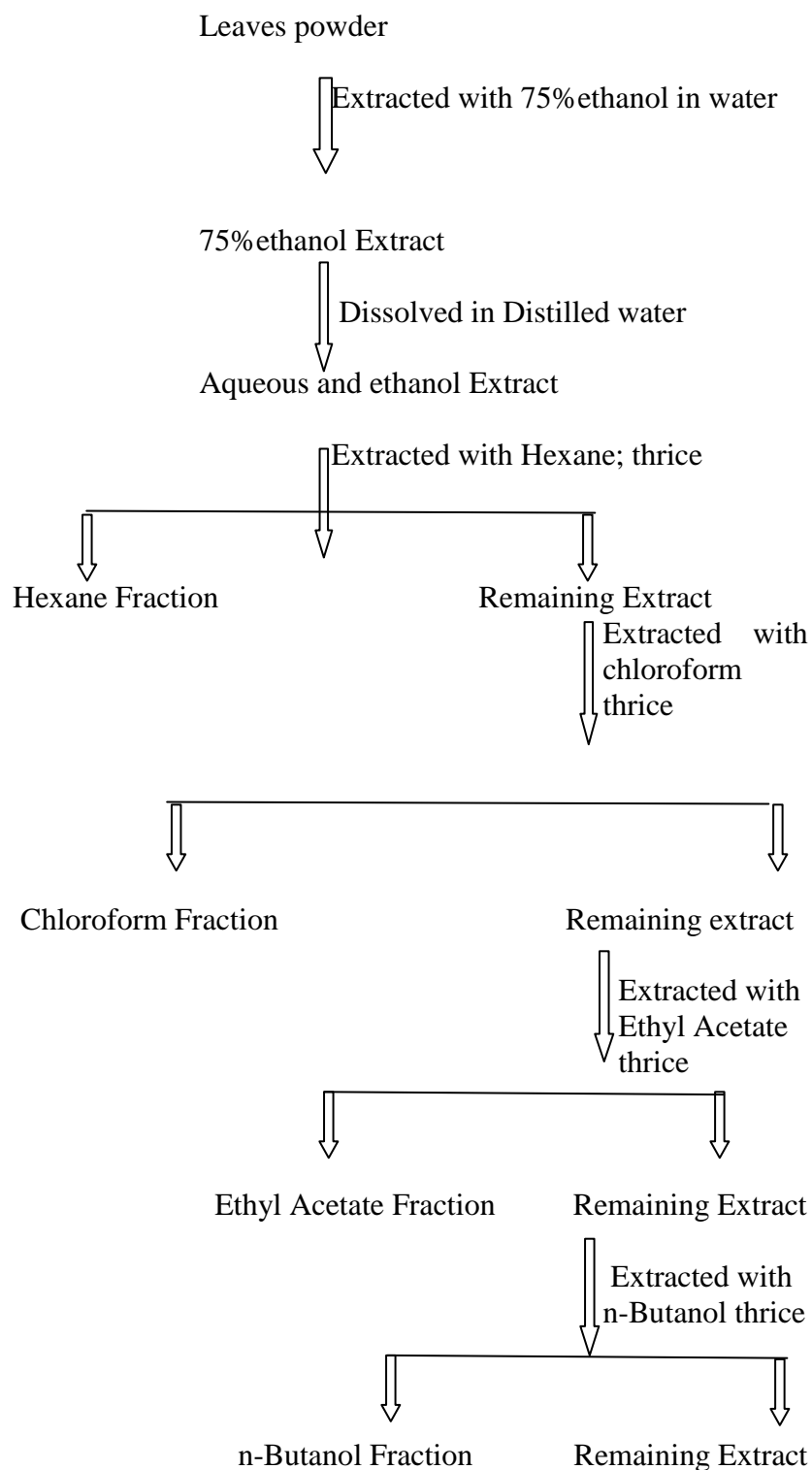
ISOLATION

Numerous beneficial effect attributed to phenolic products have given such to an interest in finding botanical species with high phenolic contents and relevant biological activity

ISOLATION OF PHENOLIC RICH FRACTION ⁶⁶

Ethanolic extract was fractionated with different solvents of increasing polarity viz hexane, chloroform, ethylacetate, n-butanol and water. Phenolic fractions obtained from the above solvents were concentrated under reduced pressure which gave a residue.

FLOW CHART OF PREPARATION OF PHENLIC RICH FRACTION



SECTION-C

TOTAL PHENOL, TOTAL TANNIN, TOTAL FLAVANOID

DETERMINATION^{67,68}

TOTAL PHENOL DETERMINATION

PRINCIPLE

Total phenolic content of the various extracts of *X. Strumarium* were determined by Folin Ciocalteu reagent method.

All the phenolic compounds are oxidised by the Folin-Ciocalteu Reagent. This reagent, which is reduced during oxidation of the phenolic substances, into a mixture of blue molybdenum and tungsten oxides.

The blue colour produced has a maximum absorption at about 750-760nm. The absorption is proportional to the quantity of oxidised phenolic compounds.

REAGENTS REQUIRED

a) Folin Ciocalteu Reagent (1N)

Commercially available Folin Ciocalteu Reagent (2N) was diluted with an equal volume of distilled water. The resultant solution was kept in a brown color bottle and stored in refrigerator at 4 °c.

b) Sodium carbonate solution (10%)

c) Standard gallic acid solution.

PROCEDURE.

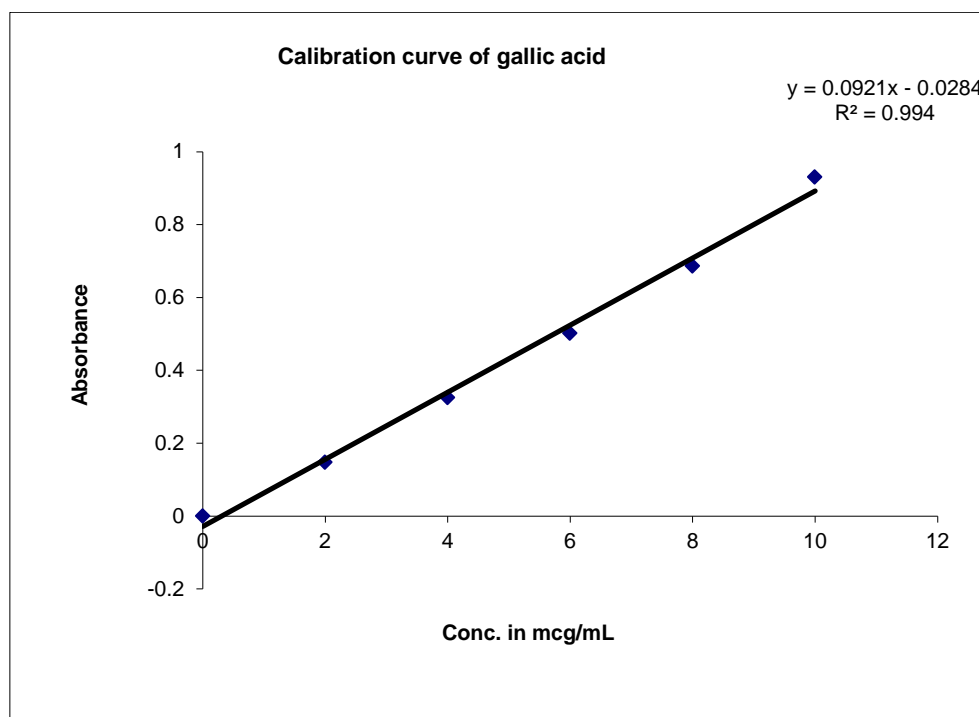
1ml of (1mg/ml) ethanolic extracts of *X. Strumarium* (EXS and EAXS) were taken in separate test tube. Add 0.5ml of Folin Ciocalteu reagent (1N) and allowed to stand for 15 mins. Then 1ml of 10% sodium carbonate solution was added. Finally the mixtures were mixed with distilled water and made upto 10ml, allowed to stand for 30 minutes at room temperature and total phenols were determined by spectrophotometrically at 760nm.

The calibration curve was generated by preparing Gallic acid at different concentration (2,4,6,8,10µg/ml). The reaction mixture without sample was used as blank. Total phenol content of various leaf extracts are expressed in terms mg of Gallic acid equivalent per gm of extract (mg GAE/g) in table 6

Table-6 ESTIMATION OF TOTAL PHENOL CONTENT OF X.
STRUMARIUM

STANDARD		ABSORBANCE	TEST		ABSORBANCE	TOTAL PHENOL CONTENT
sample	Conc.in $\mu\text{g/ml}$		Sample	Conc in $\mu\text{g/ml}$		Mg of GAE/gm of extract
Gallic acid	2	0.148 ± 0.01	EXS	100	2.535 ± 0.3	221.9 ± 0.76
	4	0.325 ± 0.03	PHENOLIC			
	6	0.502 ± 0.02	RICH			
	8	0.813 ± 0.03	FRACTION			
	10	0.823 ± 0.01	Hexane	100	2.102 ± 0.5	181.9 ± 0.6
			Chloroform	100	1.954 ± 0.3	168.7 ± 0.50
			Ethylacetate	100	3.227 ± 0.2	278.6 ± 0.49
			n-butanol	100	1.732 ± 0.3	149.6 ± 0.73

*mean of three readings \pm SEM

Fig-11: Calibration curve of Gallic acid

TOTAL TANNIN DETERMINATION^{67,69}

PRINCIPLE

Total tannin content of ethanolic extracts of *X.strumarium* was determined by Folin Denis reagent method.

Tannin like compounds reduces phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution. The intensity of which is proportional to the amount of tannins. The intensity is measured in a spectrometer at 700nm

REAGENTS REQUIRED

- a) Folin Denis Reagent (sodium tungstate 100g and phospho molybdic acid 20gm were dissolved in distilled water 750ml along with phosphoric acid 50ml. The mixture was refluxed for 2 hours and volume was made upto 1 litre with distilled water)
- b) Sodium carbonate solution (10%)
- c) Standard tannic acid solution.

PROCEDURE

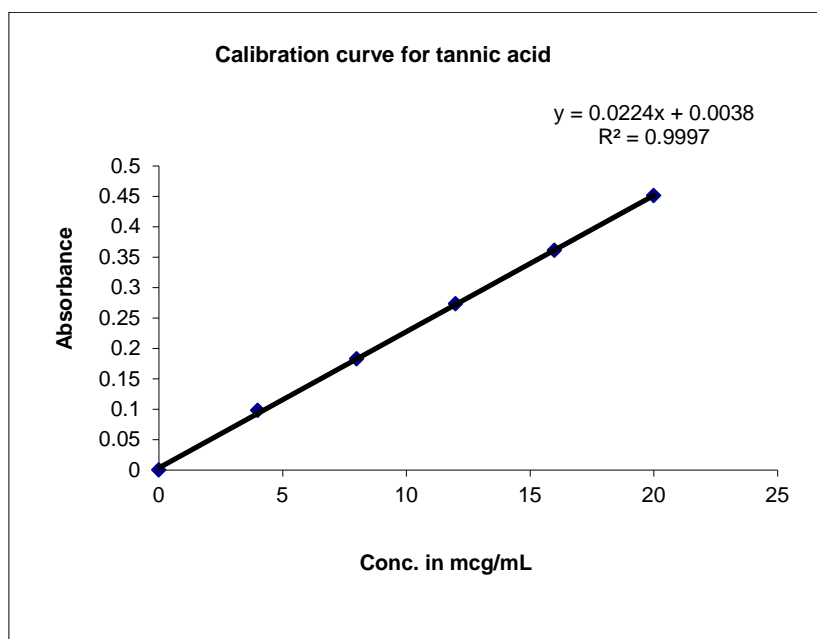
0.2ml of (1mg/ml) ethanolic extracts of *X. stramarium* was taken in a separate test tubes and mixed with distilled water to made upto the volume of 1ml. Then add 0.5ml of Folin Denis reagent and allowed to stand for 15 mins, then 1ml of sodium carbonate solution was added and the mixture was mixed with distilled water and made upto 10ml, allowed to stand for 30mins at room temperature and the tannin content was determined spectrophotometrically at 700nm.

The calibration curve was generated by preparing tannic acid at different concentration (4, 8, 12, 16, 20µg/ml). The reaction mixture without sample was used as blank. The total tannin content in the various leaf extract was expressed as milligrams of tannic acid equivalent per gm of extract.

TABLE-7 : ESTIMATION OF TOTAL TANNIN CONTENT

STANDARD		ABSORBANCE	TEST		ABSORBANCE	TOTAL TANNIN CONTENT
sample	Conc.in µg/ml		sample	Conc in µg/ml		Mg of tannic acid/gm of extract
Tannic acid	4	0.098 ± 0.02	EXS	20	0.147 ± 0.02	327 ± 0.72
	8	0.183 ± 0.01				
	12	0.203 ± 0.01				
	16	0.361 ± 0.2				
	20	0.451 ± 0.1				

mean of three readings±SEM

Fig-12: calibration curve for tannic acid

TOTAL FLAVANOIDS CONTENT ^{70,71,72}

PRINCIPLE

The aluminum chloride colorimetric technique was used for estimation of total flavonoid estimation. Aluminum ions form stable complexes with C4 keto group and either to C3 or C5 hydroxyl groups of flavones and flavonols in acidic medium. It also forms acid labile complexes with ortho hydroxyl groups in the A or B rings of flavanoids. These complexes showed a strong absorption at 415nm which is used for the estimation of flavanoids.

INSTRUMENT

Shimadzu UV Visible spectrophotometer, Model 1800

REAGENTS

10% aluminum chloride

1M potassium acetate

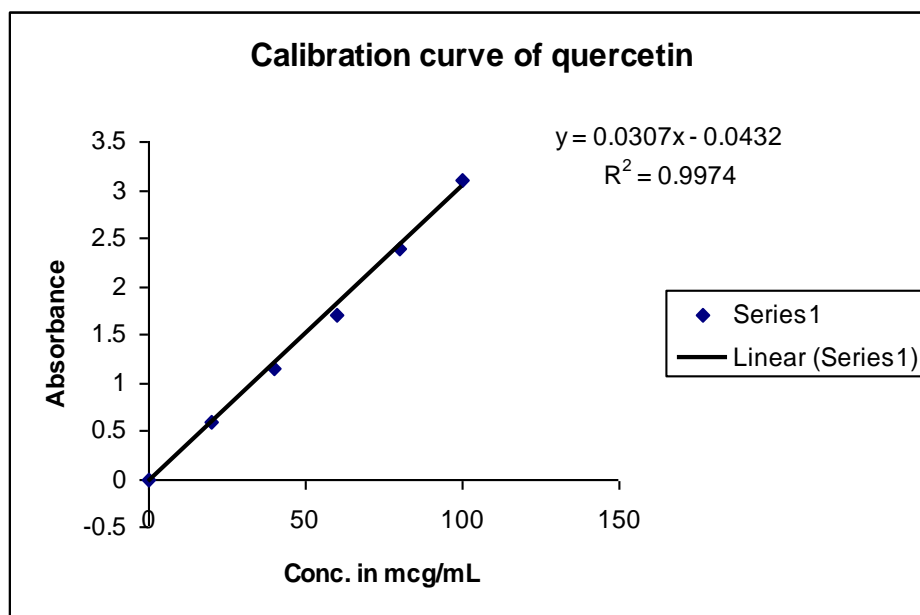
PROCEDURE

An aliquot quantity of quercetin was dissolved in ethanol to get a stock solution of 1mg/mL. Further dilutions were made to get concentrations ranging from 20-100µg/mL. 1ml of the above standard solutions were taken in different volumetric flasks, 0.1mL of aluminum chloride solution, 0.1mL of potassium acetate solution and 2.8mL of ethanol were added and the final volume was then made up to 5mL with distilled water. After 20min the absorbance was measured at 415nm. A sample without aluminium chloride was used as a blank. From the absorbance obtained, a calibration curve was constructed by plotting concentration versus absorbance of quercetin (**Fig.13**). 1mL of methanolic extract at concentrations 40µg/mL and 80µg/mL were taken and the reaction was carried out as above and the absorbance was measured at 415nm after 20min and the readings were tabulated in **Table 8**. The amount of flavonoids present can be determined by linear regression analysis. The total flavonoid content was expressed as mg quercetin equivalents /g of extract.

TABLE-8 : ESTIMATION OF TOTAL FLAVANOID CONTENT

STANDARD		ABSORBANCE	TEST		ABSORBANCE	TOTAL TANNIN CONTENT
sample	Conc.in µg/ml		sample	Conc in µg/ml		Mg of tannic acid/gm of extract
1	20	0.589 ± 0.01	EXS	100	0.027±0.02	22.015±0.5
		1.151 ± 0.04				
2	40	1.710 ± 0.09		200	0.092 ± 0.01	23.03±0.2
		2.390 ± 0.03				
3	60	3.112 ± 0.03				
4	80					
5	100					

mean of three readings±SEM

Fig: Calibration curve of quercetin

SECTION-D

TLC AND HPTLC STUDIES^{73,74}

The phytochemical evaluation of ethanolic extract of *Xanthium strumarium* and phenolic rich fraction of X.S. was carried out using TLC and HPTLC studies.

DEVELOPMENT OF CHROMATOGRAMS

The extracts were dissolved in ethanol and the spot was applied on the TLC plates using capillary tube.

The plates were developed in the chromatographic tank containing the solvent systems. Various solvent systems were tried for better results. The TLC plates were allowed to develop upto 2/3rd of the plate length and dried. The TLC plates were examined visually or under UV light.

Stationary phase - silicagel G

Mobile phase - Toluene : Ethylacetate : Formic acid
7:3:02

Detecting agent - visual & UV light

The R_f value of the spots obtained were calculated using the formula,

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

The TLC plates when examined after using different detecting agents showed the presence of different active principles in both EXS and EAXS. The spots showed

fluorescence when viewed under UV light. The active principle at R_f value 0.07 may be responsible for the antioxidant activity due to its phenolic nature.

**Table 9: Phytochemical evaluation of ethanolic extract and phenolic rich fractions
by TLC studies.**

S.NO	SOLVENT SYSTEM	EXTRACT	NO. OF SPOTS	RF VALUE	DETECTING AGENT
1.	TOLUENE : ETHYLACETATE: FORMIC ACID 7 : 3 : 0.2	EXS	3	0.7 0.8 0.9	UV - 366
		Phenolic rich fraction			
2		Hexane	3	0.71 0.81 0.89	
3		Chloroform	3	0.4 0.52 0.75	
4		Ethylacetate	3	0.39 0.63 0.72	
5		N-butanol	1	0.56	

HPTLC PROFILE:

Instrument used : CAMAG make HPTLC.

Software : winCATS 1.4.3

Sample Applicator : Linomat 5.

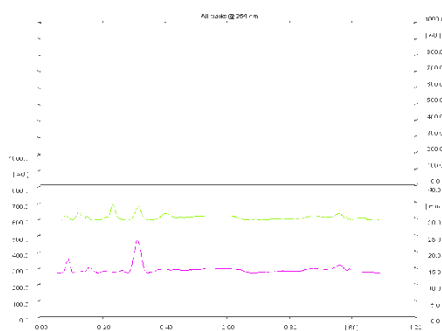
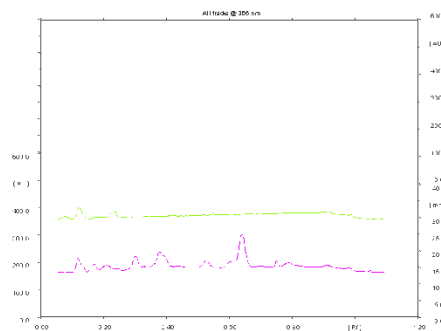
Detection : @254nm & @366nm in Densitometry TLC Scanner 3

Sample preparation : The sample was prepared in corresponding solvents

Stationary Phase : HPTLC plates silica gel 60 F 254.

Mobile Phase : Toluene: Ethyl acetate: Formic Acid (7:3:0.2)

Sample : Track 1: EXS , Track 2:EA XS

FINGERPRINTING:**3D DISPLAY****@ 254nm****@366nm**

TABLES:10 HPTLC chromatogram of both EXS and EAXS at 254nm

Peak	@ 254nm			
	Track 1		Track 2	
	Rf value	Area (AU)	Rf value	Area (AU)
1	0.09	949.1	0.08	396.8
2	0.13	192.9	0.12	714.3
3	0.16	533.6	0.15	256.0
4	0.20	160.1	0.23	1657.4
5	0.26	258.2	0.31	1543.5
6	0.31	3974.0	0.40	1317.8
7	0.40	814.9	0.60	955.4
8	0.60	1279.1	0.88	1083.6
9	0.78	457.5	0.96	1126.5
10	0.88	993.7	1.02	446.7
11	0.96	1411.4		

TABLES:11 HPTLC chromatogram of both EXS and EAXS at 366nm

Peak	@366nm			
	Track 1		Track 2	
	Rf value	Area (AU)	Rf value	Area (AU)
1	0.12	803.1	0.08	131.7
2	0.17	488.4	0.12	638.5
3	0.21	637.7	0.24	609.3
4	0.30	1241.6		
5	0.38	2419.9		
6	0.53	1135.2		
7	0.60	1011.1		
8	0.64	3053.2		
9	0.79	1370.5		

CHAPTER - 6
PHARMACOLOGICAL SCREENING
PART I
INVITRO ANTIOXIDANT ACTIVITY

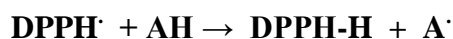
Antioxidants play an major role in the treatment of various diseases. It has been reported in many studies that the antioxidant property of the plants may due to the presence of phenolic compounds. The natural antioxidants are ascorbic acid, vitamin E, phenolic acids etc. The antioxidants scavenge the free radical generated, due to oxidative stress and exhibit a protective effect against many diseases like cancer, cardiovascular disease, diabetes and ageing. Some of the in vitro models for the evaluation of antioxidant activity are listed below⁷²

- ✓ DPPH method
- ✓ Superoxide radical scavenging activity
- ✓ Hydroxyl radical scavenging activity
- ✓ Nitric oxide radical inhibition assay
- ✓ Reducing power method
- ✓ Phosphomolybdenum method
- ✓ Peroxy nitrile radical scavenging activity
- ✓ Xanthine oxidase method
- ✓ Ferric reducing ability of Plasma
- ✓ Thiobarbituric acid assay etc.

Diphenyl picryl hydrazyl (DPPH) method ^{75,76,77}

Principle:

DPPH is a stable free radical with a distinctive ESR signal. Its reaction with antioxidants can be followed by the loss of absorbance at 517nm. It is widely accepted that DPPH accept an electron or hydrogen radical and become a stable diamagnetic molecule. Due to its odd electron, the ethanol solution of DPPH (purple colour solution) shows a strong absorption at 517nm. DPPH radicals react with suitable reducing agents where the pairing of electrons takes place and the solution loses colour stoichiometrically with the number of electrons taken up.



Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

0.1mM diphenyl picryl hydrazyl in ethanol

Procedure

A stock solution of DPPH was prepared in ethanol (4mg/100ml). To the 1mL of test samples of different concentrations (EXS and EAXS), 4mL of DPPH was added. Control without test compound was prepared in an identical manner. Blank was prepared in the similar way, where DPPH was replaced by ethanol. The reaction was allowed to be completed in the dark for about 30min. Then the absorbance of test mixtures was read at 517nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical. Vitamin C was used as standard.

The percentage scavenging was calculated using the formula

$$\% \text{inhibition} = [(\text{Control-Test})/\text{Control}] \times 100$$

The concentration of the sample required for 50% reduction in absorbance (IC₅₀) was calculated using linear regression analysis. The results obtained are presented in **Table 12** and **Fig. 15**

Nitric oxide scavenging activity assay^{78,79}

Principle

Nitric oxide scavenging activity was determined according to the method reported by Green *et al.*, 1982 [78]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interact with oxygen to produce nitrite ions. These nitrite ions can be determined by Griess Illosvoy reaction. The nitrite ions produced diazotizes sulphanilamide and the diazonium salt thus obtained reacts with NN naphthyl ethylene diamine dihydrochloride to give a pink colour chromophore which has a maximum absorption at 546nm.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

10mM sodium nitroprusside

Phosphate buffered saline pH 7.4

2% sulphanilamide in ortho phosphoric acid

0.1% naphthyl ethylene diamine dihydrochloride

Procedure

To 1mL of sodium nitroprusside, 2.5mL phosphate buffered saline pH 7.4 was added. 1mL of extracts at various concentrations were added to the above solution and the mixture was incubated at 25°C for 30min. To 1.5mL of the incubated mixture add 1mL of sulphanilamide in phosphoric acid and 0.5mL of naphthyl ethylene diamine dihydrochloride. The absorbance was measured at 546nm. Ascorbic acid was

used as a standard. The percentage inhibition of nitric oxide radical generated was calculated using the following formula:

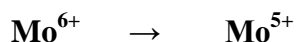
$$\% \text{ inhibition} = [(\text{Control-Test})/\text{Control}] \times 100.$$

The IC₅₀ was calculated using linear regression analysis. The results were presented in **Table 13** and **Fig. 16**

Total antioxidant activity by phosphomolybdenum method ⁸⁰

Principle

This is a spectrophotometric method. The assay is based on the reduction of Mo(VI) to Mo(V) by the sample and by the subsequent formation of green phosphate Mo(V) complex at acidic pH which has a maximum absorption at 695nm. This method is routinely used to determine total antioxidant activity of samples.



Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

0.6M sulphuric acid

28mM sodium phosphate

4mM ammonium molybdate

Procedure

An aliquot of 0.3ml of different concentrations of sample solutions was combined with 2.7ml of the reagent solution (H₂SO₄, sodium phosphate and ammonium molybdate). 0.3mL of methanol was used in place of sample for control. The tubes were incubated in a boiling water bath at 95°C for 90min. After the samples had cooled to room temperature, the absorbance was measured at 695nm against

blank. The standard vitamin C was treated in a similar manner. The antioxidant activity was expressed as equivalents of Vitamin C ($\mu\text{g/g}$). The results are tabulated in **Table14** and **Fig.17**

Table 12: Free radical scavenging activity of EXS, EAXS by DPPH assay

S. No.	Conc. in $\mu\text{g/mL}$	Percentage inhibition by standard ascorbic acid	Percentage inhibition by EXS	Conc. in $\mu\text{g/mL}$	Percentage inhibition by EAXS
1	20	25.86	38.56	5	43.86
2	40	53.32	47.21	10	54.24
3	60	62.2	59.76	15	65.87
4	80	81.21	65.42	20	73.36
5	100	93.73	78.12	25	85.20
	IC_{50}	47.06 $\mu\text{g/mL}$	52.65 $\mu\text{g/mL}$	IC_{50}	11.25 $\mu\text{g/mL}$

Fig. 15: Free radical scavenging of EXS against DPPH

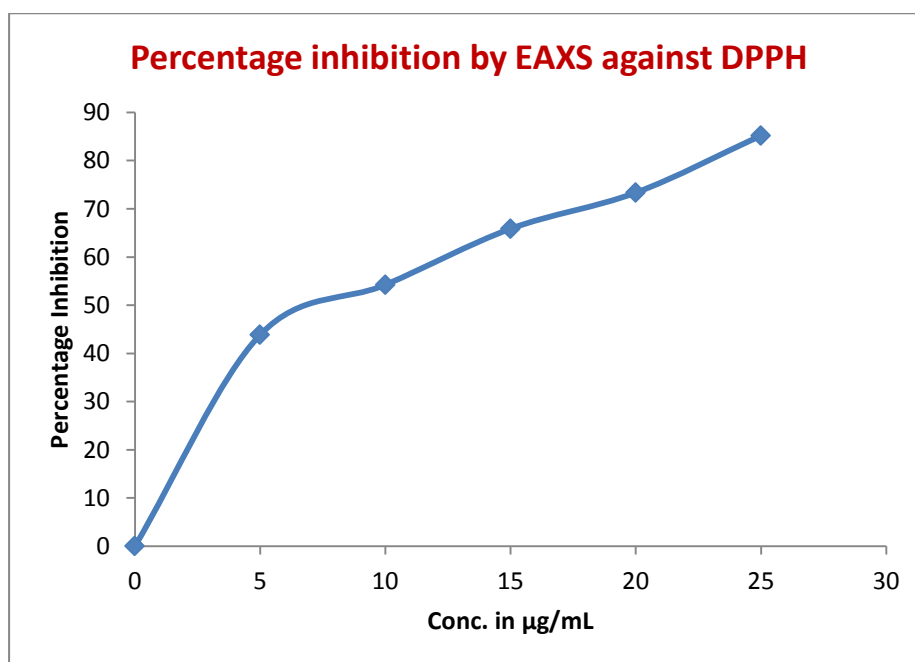
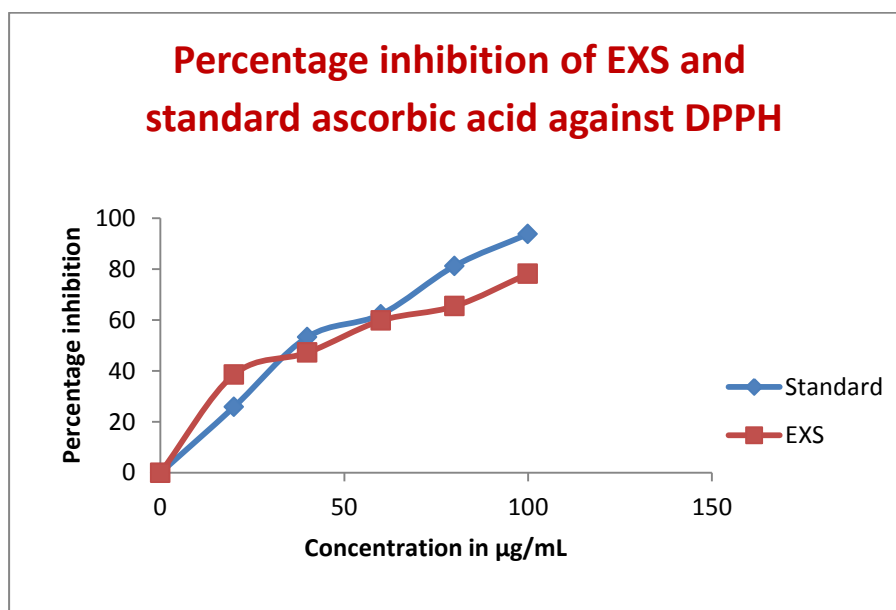


Table 13: Percentage inhibition of ethanolic extract of *X. strumarium* against nitric oxide.

S. No.	Conc. in $\mu\text{g/mL}$	Percentage inhibition by standard ascorbic acid*	Percentage inhibition by EXS*
1	44.44	55.71 ± 0.45	36.51 ± 2.39
2	88.89	58.94 ± 0.38	49.77 ± 1.50
3	133.33	76.61 ± 3.13	65.01 ± 0.21
4	177.78	78.27 ± 2.56	70.39 ± 0.04
5	222.22	80.03 ± 1.97	74.08 ± 0.38
	IC_{50}	$84.64 \mu\text{g/mL}$	$113.48 \mu\text{g/mL}$

*mean of three readings \pm SEM

Fig. 16: Nitric oxide radical scavenging by ethanolic extract of *X.strumarium*

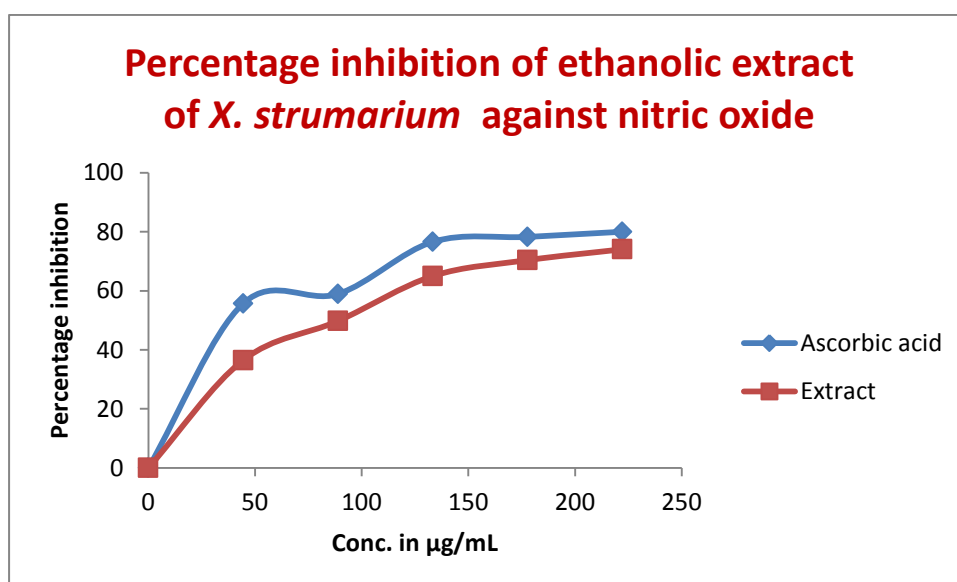
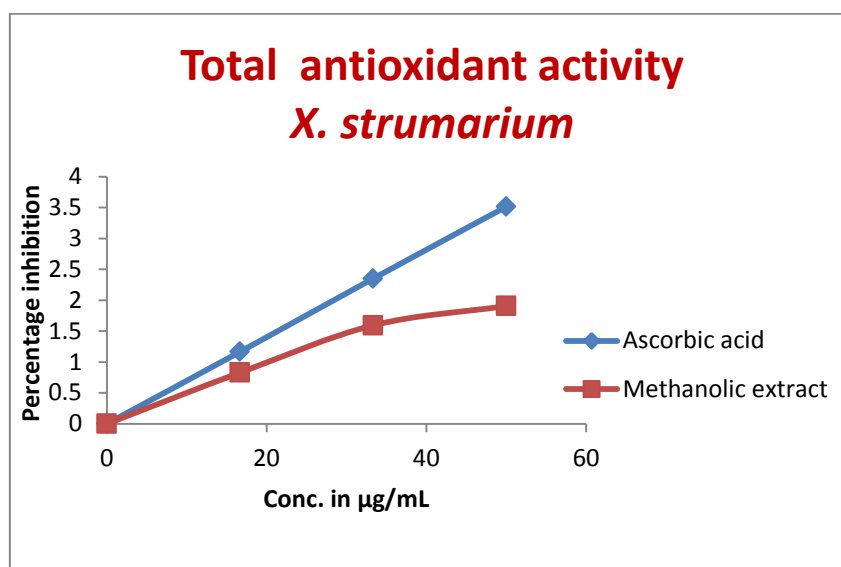


Table 14: Total antioxidant activity of Ethanolic extract of *X. strumarium*

S.No.	Conc. in $\mu\text{g/mL}$	Abs. of standard ascorbic acid*	Conc. in $\mu\text{g/mL}$	Abs. of EXS*
1	16.67	1.165 ± 0.006	16.67	0.828 ± 0.008
2	33.33	2.350 ± 0.001	33.33	1.593 ± 0.003
3	50	3.515 ± 0.004	50	1.906 ± 0.007

*mean of three readings \pm SEM

Figure17 : Total antioxidant activity of Ethanolic extract of *X. strumarium*



PART- 1I

CARDIOPROTECTIVE EFFECT OF LEAF EXTRACT OF *XANTHIUM STRUMARIUM* ON DOXORUBICIN INDUCED CARDIOTOXICITY IN RATS ^{81,82,83,84,85}

Cardiotoxicity was induced in rats using doxorubicin. Though doxorubicin is a potential antineoplastic drug its therapeutic spectrum is narrowed due to its cardiotoxic side effects. Administration of the doxorubicin at 15mg/kg body weight of albino rats leads to cardiomyopathy and heart failure.

MATERIALS AND METHODS:

ANIMAL:

Male albino rats (150-200g) were procured from the central animal house, Institute of Pharmacology, Madurai medical college, and also from an approved supplier (License No:) The animals were housed under standard conditions of temperature ($25^{\circ} \pm 2^{\circ}$ C) and photoperiod of 12 hours dark/light cycle with food and water at libidum. The institutional animal ethical committee clearance was obtained and the experimental study was conducted according to the protocol approved by them.

CHEMICALS AND REAGENTS

- ✓ CK-MB DIAGNOSTIC KIT
- ✓ LDH DIAGNOSTIC KIT
- ✓ TRIS HCL BUFFER
- ✓ THIOBARBITURIC ACID (TBARS)
- ✓ DTNB REAGENT

CARDIOTOXICITY INDUCING AGENT:

Doxorubicin (DOXIGLAN), the cardiotoxic agent was purchased from Gland pharma limited. This drug was reconstituted with sterile water for injection and used for this study. Doxorubicin at a dose of 15mg/kg was administered intraperitoneally to rats in order to reduce cardiotoxicity.

EXPERIMENTAL PROTOCOL:

The animals were divided into 5 groups and each group consists of six animals (n=6). Group I and II animals were administered 1% CMC alone while group III and IV animals were administered with ethanolic extract of *Xanthium strumarium* - EXS (as a suspension in 1% CMC) at 100mg/kg and 200mg/kg doses respectively. Group V animals were administered the phenolic rich fraction of *Xanthium strumarium* - EAXS (10mg/kg) for five consecutive days by oral route. On the fifth day, group III, IV, V animals were administered extracts an hour prior to the administration of doxorubicin

GROUP	TREATMENT
I	1% CMC in distilled water for five consecutive days orally
II	Single dose for Doxorubicin at 15mg/kg IP was administered on fifth day alone.
III	EXS at 100mg/kg PO was administered for consecutive days and Doxorubicin 15mg/kg IP on the fifth day alone.
IV	EXS at 200mg/kg PO was administered for five consecutive days and Doxorubicin 15mg/kg IP on the fifth day alone

V EXS at 200mg/kg PO was administered for consecutive days and Doxorubicin 15mg/kg IP on the fifth day alone.

After 24 hours of doxorubicin administration blood samples were collected. All the animals were sacrificed as per the CPCSEA guidelines. Heart was isolated and used for Histopathological and antioxidant studies.

PARAMETERS EVALUATED:

- **Serum parameters**
CK - MB and LDH
- **Estimation of invivo antioxidants in cardiac tissue homogenate**
TBARS, GSH and Total protein.
- Histopathological studies.

ESTIMATION OF INVIVO ANTIOXIDANTS

PREPARATION OF TISSUE HOMOGENATE

The isolated heart was washed and blotted in a filter paper. A 10% tissue homogenate was prepared by using chilled 0.15M Tris Hcl (P^H 7.4) buffer and centrifuged at 5000rpm for 5minutes. The clear supernatant liquid was used for the estimation TBARS, GSH and total protein.

ESTIMATION OF LIPID PEROXIDATION (TBARS):

Lipid peroxidation was estimated by the method described by Rajkumar D.V. et al.

To 1ml of the tissue homogenate, 2.5ml of trichloroacetic acid (20%) were added and centrifuged. 2.5ml of 0.05M sulphuric acid and 3.5ml thiobarbituric acid were added to the supernatant liquid and incubated at 37°C for 30minutes. It was then extracted with n-butanol. Intensity of the chromogen in the layer was measured at 530nm using UV spectrophotometer. The were presented in the **table 16. Fig 19**

ESTIMATION OF REDUCED GLUTATHION:

Reduced Glutathione was estimated by the method described by Morn et al.,(1979)

1 ml of tissue homogenate was precipitated with 1 ml of 10 % TCA and was centrifuged to remove the precipitate. To 0.5 ml of the supernatant added 2 ml of 0.6 mM 5,5'Dithiobis-2-nitrobenzoic acid(DTNB) in 0.2 M Sodium phosphate and the total volume was made upto 3 ml with 0.2 M Phosphate buffer (pH 8). The absorbance was read out at 412nm. The results obtained were presented in **table 16.**

Fig 19

Table 15: Effect of EXS and EAXS on serum biochemical parameters

Groups	CK-MB	LDH
--------	-------	-----

	IU	IU
Control	126.08 \pm 0.76	106.266 \pm 0.77
Doxorubicin	292.03 \pm 0.77	216.53 \pm 0.68
EXS(100)	203.03 \pm 0.8*	176.65 \pm 0.85*
EXS(200)	134.98 \pm 1.04*	133.15 \pm 0.79*
EAXS(10)	130.01 \pm 0.6*	124.81 \pm 1.10*

Values expressed as mean SEM *p<0.001 when compared with Doxorubicin

Digits in parentheses indicate dose in mg/kg

CK-MB – Creatine kinase - MB, LDH – Lactate dehydrogenase

EXS Ethanollic extract(75%) of *Xanthium strumarium*

EAXS Ethylacetate fraction of *Xanthium strumarium*.

Table 16: Effect of EXS and EAXS on tissue biochemical parameters.

Groups	TBARS nmoles/g protein	GSH nmoles/g protein
Control	60.15 \pm 0.67	2.86 \pm 0.3
Doxorubicin	129.11 \pm 0.76	5.01 \pm 0.2
EXS(100)	104.96 \pm 1.1*	2.68 \pm 0.2*
EXS(200)	81.98 \pm 0.91*	2.38 \pm 0.1*
EAXS(10)	75.55 \pm 0.9*	2.06 \pm 0.2*

Values expressed as mean SEM *p<0.001

TBARS – Thiobarbituric acid

GSH - Reduced glutathione

HISTOPATHOLOGICAL STUDIES:

The hearts isolated from each group were preserved in 10% formalin, processed and embedded in paraffin blocks. A four μm thick paraffin section were cut and stained with eosin and hematoxylin over a glass slide. They were observed under light microscope and the pathological changes were recorded.

STATISTICAL ANALYSIS:

All the datas were expressed as standard error of mean (SEM). Data of biochemical parameters were analysed using one way ANOVA. Tukeys's multiple range test was applied for post-hoc analysis. A value of $p < 0.001$ was considered to be statistically significant.

PART III

INVITRO ANTIARTHRITIC ACTIVITY ⁸⁶⁻⁹¹

Rheumatoid arthritis is an autoimmune disorder. One among the cause for the disease is due to the denaturation of the protein. Antiarthritic activity was studied by inhibition of protein denaturation method .

MATERIALS REQUIRED

Test Extracts	EXS(Ethanolic extract of <i>X.Strumarium</i>) and EAXS(ethylacetate fraction of <i>X.strumarium</i>)
Standard Drug	Diclofenac sodium(250 µg/ml)
Chemicals And Solutions	Bovine serum albumin (5% w/v aqueous solution) Phosphphate buffer (P ^H 6.3)
Instrument	UV/ Visible spectrophotometer at 416 nm.

EXPERIMENTAL PROTOCOL

The following four solutions were prepared

1. Test solution (0.5ml)

The test solution consists of 0.45ml bovine serum albumin (5% w/v aqueous solution) and 0.05ml of test solution (100, 200, 400, 800, 1000 µg/ml concentrations of EXS (or) 50, 100, 150, 200, 250 µg/ml concentrations of EAXS)

2. Test control solution (0.05ml)

The test control solution consists of 0.45ml bovine serum albumin and 0.05ml distilled water.

3. Product control (0.5ml)

The product control consists of 0.45ml distilled water and 0.05ml test solution (of different concentrations).

4. Standard solution (0.5ml)

Standard solution consists of 0.45ml of bovine serum albumin and 0.05ml of Diclofenac sodium solution (250µg/ml).

All the above test samples was adjusted to p^H 6.3 using a small amount of 1N HCL. They were incubated at 37⁰C for 20 minutes and heated at 57⁰ C for 3 minutes. Allow to cool and about 2.5ml of phosphate buffer (p^H 6.3) was added to all the above solution. The absorbance was measured using UV spectrophotometer at 416nm. The percentage inhibition of protein denaturation was calculated using the formula.

$$\text{Percentage inhibition} = 100 - \frac{\{ (\text{OD of test solution} - \text{OD of product control}) \}}{\text{OD of test control}}$$

The control represents 100% protein denaturation. The results were compared with the standard drug, diclofenac sodium (250µg/ml) treated sample. The results were presented in table 17, fig 20.

Table 17 Effect of EXS and EAXS on Inhibition of Protein Denaturaion

S.NO	% inhibition			
	Conc. in µg/ml	EXS	Conc. in µg/ml	EAXS
1	100	55.62 ± 2.65	50	64.76 ± 2.68
2	200	62.28 ± 3.74	100	68.21 ± 1.39
3	400	76.59 ± 1.45	150	73.53 ± 4.15
4	800	81.77 ± 2.89	200	77.92 ± 2.57
5	1000	85.98 ± 4.37	250	82.56 ± 5.90

*mean of three readings ± SEM

PART IV

ANTIBACTERIAL ACTIVITY AGAINST RHEUMATIC FEVER AND ENDOCARDITIS^{92,93,94}

Ethanollic extract of *xanthium strumarium* and its ethylacetate fraction of *xanthium strumarium* were screened for the antibacterial effect against the microorganisms causing rheumatic fever and endocarditis.

MATERIALS REQUIRED

Medium

Blood agar medium was used for *Streptococi pyrogenes* and Muller Hinton Agar (MH, Hi media) was used for *Streptococci viridians* and *Enterococci faecalis*.

Microorganisms

- *Streptococi pyrogenes*
- *Streptococci viridians*
- *Enterococci faecalis*

Drugs

Ethanollic extract of X.S (EXS) and Ethyl acetate fraction of X.S (EAXS).Penicilin was used as the standard drug.(dose)

EXPERIMENTAL PROCEDURE

Disc Diffusion technique

The pathogenic strains were then seeded on the blood agar and MH media in a petridish by streaking the plate with the help of a sterile swab. Care was taken for the even distribution of culture all over the plate. The seeded plates were allowed to dry

Test Procedure

The plain sterile discs of 6mm diameter were obtained from Hi Media. The discs were then impregnated with different concentrations of the EXS and EAXS in

solvent DMSO. Penicillin disc was used as the standard. Each disc contained 30µg. The penicillin and extract discs were then placed on the seeded medium plates. The plates were then incubated at 37°C for 24h. The results were read by the presence or absence of zone of inhibition. The zone of inhibition was measured. The results were tabulated in **table 18s**

Table 18 Anti – bacterial activity of EXS and EAXS

S.no	Organism	STD		EXS		EAXS	
		Conc in µg	Zone of inhibition (mm)	Conc in mg	Zone of inhibition (mm)	Conc in mcg	Zone of inhibition (mm)
1	<i>Streptococcus pyrogenes</i>	30	22	2	2	100	8
				4	8	200	14
				6	12	300	20
2	<i>Streptococcus viridians</i>		25	2	-	100	10
				4	4	200	16
				6	12	300	22
3	<i>Enterococcus faecalis</i>		26	2	-	100	8
				4	4	200	15
				6	12	300	22

CHAPTER 7

RESULT AND DISCUSSION

Xanthium strumarium linn (Asteracea) commonly known as cocklebur is a weed distributed throughout India. Various phytoconstituents has been reported in this plant such as flavanoids, tannins, phenols. This plant has been reported to possess diuretic, antidiabetic, antimalarial, cytotoxic, antibacterial and antifungal activities. This dissertation work includes the pharmacognostical ,phytochemical and pharmacological studies on the leaves of *Xanthium strumarium*.

Chapter 1

Introduction of this dissertation work has been discussed in this chapter which gives information about herbal plants, its safety, pharmacovigilance, quality control and scientific validation. A brief note on antioxidants, heart failure, its etiology, clinical management, cardiomyopathy, cardiotoxicity, cardiotoxic agents, herbal remedies used as cardioprotective agent were discussed in this chapter. Rheumatoid arthiritis, its sign and symptoms, diagnosis, treatment, herbal drugs used in the treatment of this disease. Rheumatic fever, its cause, mechanism and the treatment have also been discussed in this chapter.

Chapter 2

Literature survey pertaining to the pharmacognostical, phytochemical, pharmacological studies of *Xanthium strumarium* were discussed in this chapter. The Ethnomedical information revealed that the plant is used for the treatment of rheumatoid

arthritis, cardiac problems, kidney diseases, malaria, diabetes, tumour and also possess antibacterial and antifungal activity.

Chapter 3

Aim and scope of the present work has been discussed in this chapter considering the ethnomedical information and chemical nature of phytoconstituents present in this plant.

Chapter 4

This chapter deals with the pharmacognostical studies on the leaves of this plant. The systematic position, habit & habitat and description of the plant were discussed in this chapter. An attempt has also been made to standardize the leaves of this plant by means of organoleptic, microscopical and physical evaluation and the results were tabulated.

Macroscopical studies

The leaves are cordate or sub – orbicular in shape, irregular toothed margin and cuneate apex.

Microscopical studies

The following anatomical characters were found in the T.S of the leaves. The leaf consists of very thick midrib and thin lamina. Actinocytic stomata are present on both adaxial and abaxial part of the epidermis. Both glandular and non glandular trichomes are present on the sides of the lamina and the glandular trichomes are not much prominent. Non glandular trichomes are 2-5 celled uniseriate, unbranched and thin walled. Glandular trichomes are small, delicate, dumbbell shaped and two celled. They are situated in the shallow pits of the epidermis. Parenchymatous ground tissue also

consists of circular secretory cannals with a ring of epithelial cells. Vascular system of the midrib is multistranded. About five bundles are arranged in a shallow arc along the abaxial part and two bundles are located in the adaxial part.

Petiole consists of basal, middle and terminal part. It consists of three epidermal layers followed by four or five layers of collenchymas cells and rest of the ground tissue consists of large thin walled parenchyma cells. Secretory canals with epithelial cells are scattered in the ground tissue. Vascular system exhibits much variation in the three regions of the petiole.

Powder microscopy

The powder microscopy of the leaves showed the following distinct features.

- ✚ Epidermis with actinocytic stomata.
- ✚ Multicellular, uniseriate and unbranched non glandular trichlomes.
- ✚ Delicate dumbbell shaped, two celled glandular trichlomes are rarely seen.
- ✚ Secretory cells surrounded by epithelial cells along with parenchyma cells.
- ✚ Liginfied, spiral xylem vessels.

Quantitative microscopy

Leaf constants such as stomatal number, stomatal index, vein islet, vein termination were determined for the leaves of this plant and the results were tabulated (table 1)

The physical parameters like ash value (total ash, water soluble ash, acid insoluble ash), loss on drying and extractive value were determined for various solvents and the results were presented in the table (table 2 and 3)

Ethanol extract (75%) showed highest extractive value (6.44 %). When compared to ethanolic extract(95%) (2.33%), chloroform extract (4.01%), petroleum ether extract (0.47%) and the results were presented in table.

Table 1: Quantitative microscopical parameters of the leaf of *Xanthium strumarium*.

S. No.	Parameters*	*Values obtained
1	Stomatal number in upper epidermis	103.26 \pm 0.12
2.	Stomatal number in lower epidermis	227.43 \pm 0.32
3.	Stomatal index in upper epidermis	17.63 \pm 0.34
4.	Stomatal index in lower epidermis	26.54 \pm 0.63
5.	Vein islet number	13.49 \pm 0.13
6	Vein termination number	2.63 \pm 0.24

* mean of three readings

Table 2: Analytical parameters of *X.strumarium*

S. No	Parameters*	Values* expressed as %
1	Ash values	
	Total ash	11.42 \pm 1.34
	Water insoluble	6.57 \pm 0.95
	Water soluble ash	4.85 \pm 0.74
	Acid insoluble ash	2.49 \pm 0.62
2	Loss on drying	2.14 \pm 0.31

* mean of three readings

Table 3: Extractive values of *X.strumarium*

S. No	Parameters*	Values* expressed as %
	Extractive values	
1	Petroleum ether extract	0.4765
2	Ether extract	3.517
3	Hexane	3.318
4	Chloroform	4.015
5	Acetone	1.559
6	Methanol	3.289
7	Ethanol	2.335
8	75% Ethanol	6.437
9	Aqueous	5.218

Chapter-4

Collection, authentication and preparation of plant extract have been discussed in this chapter. Both powdered leaf material and various extracts were subjected to preliminary phytochemical studies which indicates the presence of flavanoids, tannin, phenols, alkaloids and saponins. The phytochemical study also revealed that ethanolic extract(95%) showed the presence of almost all the phytoconstituents viz. Flavanoids, tannin, phenols, and alkaloids and these phytoconstituents also reported to possess significant therapeutic potential for the treatment of various diseases. Hence ethanolic extract(75%) showed highest extractive value (6.44%) and therapeutically viable

phytoconstituents are also present. So it has been chosen for the phytochemical and pharmacological studies

This part of the study deals with extraction of plant materials with ethanol(75%) by triple maceration. Preparation of phenolic rich extract from ethanolic extract of X.S by fractionation using hexane, chloroform, ethylacetate and n-butanol were discussed in this chapter.

Ethylacetate fraction showed highest phenolic content (278.6mg/kg) compared to hexane(181.96mg/kg), chloroform(168.76mg/kg), n-butanol (149.66mg/kg) fractions. This study revealed that the total phenolic content was found to be 221.96mg/kg and 278.66mg/kg for EXS and EAXS respectively

This study indicate that the ethylacetate rich fraction exhibited highest phenolic content when compared to other fractions and also revealed that both EXS and EAXS showed significant concentration of phenolic compounds.

Total phenol, total tannin and total flavanoid content

Extracts	Total phenol content mg/g	Total tannin content mg/g	Total flavanoid content mg/g
EXS	221.9 ± 0.76	327 ± 0.72	23.03 ± 0.2
Phenolic rich extract			
Hexane	181.9 ± 0.6	-	-
Chloroform	168.7 ± 0.50	-	-
Ethylacetate	278.6 ± 0.49	-	-
n-butanol	149.6 ± 0.73	-	-

*** mean of three readings**

TLC and HPTLC studies

This chapter deals with the TLC and HPTLC studies on EXS and EAXS. EXS and all the phenolic fractions were subjected to TLC studies which revealed that EXS, ethylacetate, chloroform and hexane fraction showed three spots each and n-butanol fraction exhibited only one spot

HPTLC chromatogram was recorded for both EXS and EAXS. HPTLC chromatogram of EXS indicate that about eleven peaks were obtained at 254nm and nine peaks were obtained at 366nm. HPTLC chromatogram of EAXS showed that about ten

peaks were obtained at 254nm and three peaks were obtained in 366nm. This HPTLC study also showed that about eleven phytoconstituents were present in EXS and ten phytoconstituents were present in EAXS.

Table 12: Phytochemical evaluation of ethanolic extract and phenolic rich fractions by TLC studies.

S.NO	SOLVENT SYSTEM	EXTRACT	NO. OF SPOTS	RF VALUE	DETECTING AGENT
1.	TOLUENE : ETHYLACETATE: FORMIC ACID 7 : 3 : 0.2	EXS	3	0.7 0.8 0.9	UV - 365
		Phenolic rich fraction			
2		Hexane	3	0.71 0.81 0.89	
3		Chloroform	3	0.4 0.52 0.75	
4		Ethylacetate	3	0.39 0.63 0.72	
5		N-butanol	1	0.56	

Chapter 5

This chapter deals with the pharmacological screening of both EXS and EAXS for invitro antioxidant, cardioprotective, anti- arthritic and anti-bacterial activity.

Part I

Radical scavenging activity was determined for EXS and EAXS by DPPH assay. The percentage inhibition of EXS (100µg/ml) and EAXS (25µg/ml) were found to be 78% and 85% respectively when compared to the standard ascorbic acid 93.73% (100µg/m). IC₅₀ of EXS and EAXS were found to be 52.65µg/ml and 11.25µg/ml respectively when compared to the standard ascorbic acid (47.04µg/ml). This study showed that both EXS and EAXS exhibited significant radical scavenging activity but EXS has also shown more potent activity than ascorbic acid.

Nitric oxide scavenging was determined for EXS. At 222µg/ml percentage inhibition of EXS and ascorbic acid were found to be 72.12% and 80.03% respectively. EXS and ascorbic acid showed the IC₅₀ value of 113.48µg/ml and 84.64µg/ml respectively. This study revealed that EXS showed significant nitric oxide scavenging inhibition assay when compared to ascorbic acid.

EXS was also determined for the total antioxidant potential by phosphomolybdenum method. Total antioxidant potential of EXS was found to be 1.906 (200 µg/ml) when compared to ascorbic acid 3.515 (15 µg/ml). This study revealed that EXS exhibited significant total antioxidant potential when compared to ascorbic acid.

Part II

This part of pharmacological study is concerned with the evaluation of cardio protective effect of EXS and EAXS on Doxorubicin induced cardiotoxicity in rats. This study involves the estimation of biochemical parameters in the serum such CK-MB, LDH and TBARS, GSH in the heart tissue homogenate of the rat.

Doxorubicin treated animals, the serum LDH ($p<0.001$) and CK-MB ($p<0.001$) were increased significantly when compared to the serum parameters of the control animals. It was also found that, there was significant increase in TBARS level ($p<0.001$) and significant decrease in GSH level ($p<0.001$) as compared to the tissue parameters of the control animals.

At 100mg/kg EXS treatment, the serum LDH ($p<0.001$) and CK-MB ($p<0.001$) were found to be decreased when compared to the serum parameters of the Doxorubicin treated animals. The TBARS level ($p<0.001$) was decreased and GSH level ($p<0.001$) was elevated compared to the tissue parameters of the doxorubicin treated animals.

At 200mg/kg EXS treatment, the serum LDH ($p<0.001$) and CK-MB ($p<0.001$) level were significantly decreased as compared to the serum parameters of the Doxorubicin treated animals. The TBARS level ($p<0.001$) was significantly decreased and GSH level ($p<0.001$) was significantly increased as compared to the tissue parameters of the doxorubicin treated animals.

At 10mg/kg EAXS, the serum LDH ($p<0.001$) and CK-MB ($p<0.001$) level were decreased significantly when compared to the serum parameters of the Doxorubicin treated animals. The TBARS level ($p<0.001$) was decreased significantly and GSH level

($p < 0.001$) was increased significantly when compared to the tissue parameters of the doxorubicin treated animals

Histopathological studies

In the histopathological studies, the control animals showed normal myocardial structure. The incidence of marked cytoplasmic vacuoles, nuclear pyknosis and dilated blood vessels were observed in Doxorubicin treated animals. Treatment with 100 mg/kg EXS, 200mg/kg EXS and 10 mg/kg EAXS showed no significant pathological changes and the extract possess cardioprotective activity against Doxorubicin.

Fig 18 Effect of EXS and EAXS on serum biochemical parameters

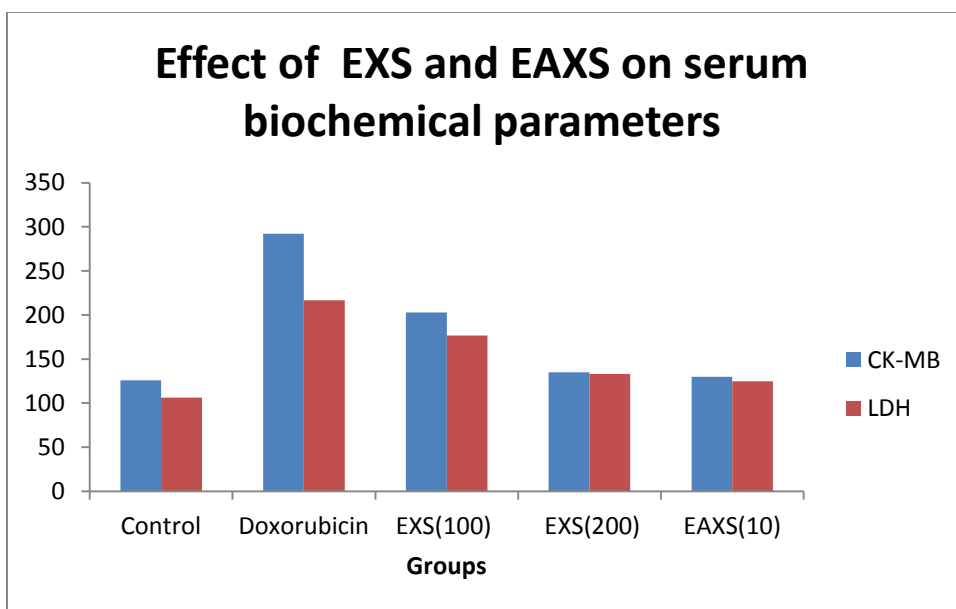
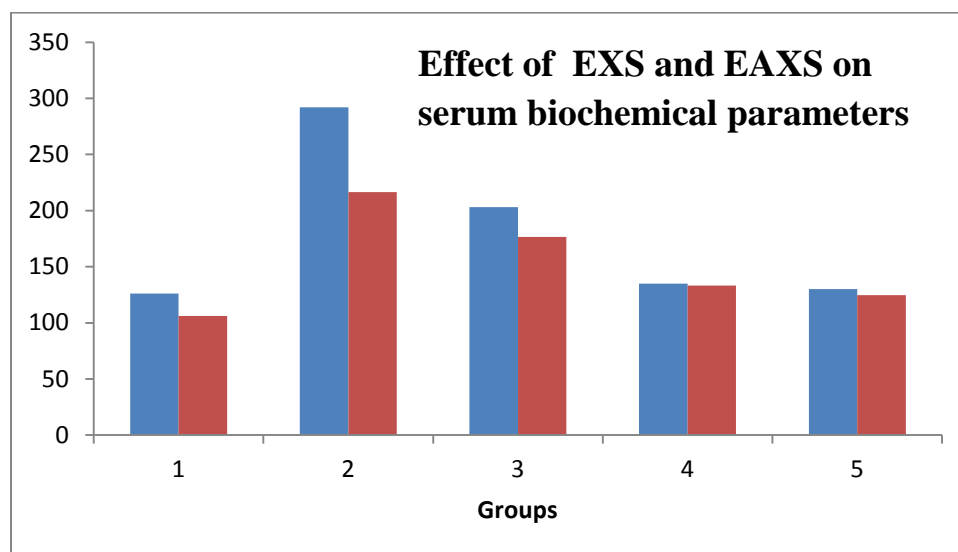


Fig19 Effect of EXS and EAXS on tissue biochemical parameters

Discussion

Doxorubicin, a powerful antineoplastic agent causes systolic dysfunction and ventricular arrhythmia in a dose dependent manner ($>450\text{mg/m}^2$ and frequent with $>550\text{mg/m}^2$). By measuring the cardiac specific troponin and monitoring by echocardiography, it was found that Doxorubicin administration deteriorated LV function of the heart.

The development of these complications appears to be related to the oxidative stress, free radical generation, damage of inner mitochondrial membrane and interference with the synthesis of adenosine triphosphate (ATP)

Doxorubicin possess quinone and hydroquinone moieties. Doxorubicin undergoes one electron reduction to form intermediate semiquinone radical which in turn reacts with oxygen to form superoxide anion radicals. This superoxide anion radical is capable of generating both hydrogen peroxide and hydroxyl radical which attacks DNA and oxidizes DNA bases.

Oxygen radical induced injury in the lipid membrane was considered to be responsible for Doxorubicin induced cardiotoxicity. Oxidative stress, lipid peroxidation and generation of free radical induced by doxorubicin leads to increased CK-MB, LDH, TBARS level and decreased GSH level. EXS and EAXS treated animals resulted elevation of endogenous GSH and reduction of CK-MB, LDH and TBARS.

The leaf has been reported for the presence of tannins. It is evident that polyphenolic compounds (flavanoids, tannins) were present in both EXS and EAXS. Both EXS(221.9mg/g) and EAXS(278.6mg/g) were estimated for the total phenolic content which revealed that these extracts consists of phenolic compounds significantly.

Preliminary phytochemical studies indicate presence the phenolic constituents such as flavanoids, tannins in EXS. Quantification of EXS for total flavanoids, total phenol and total tannin content showed the highest concentration of phenolic constituents.

Both EXS and EAXS showed potential invitro antioxidant activity by DPPH assay, Nitric oxide scavenging activity and phosphomolybdenum method. Mechanism behind the cardiotoxicity is free radical generation and oxidative stress. These plant extract also exhibited invivo antioxidant activity by elevating endogenous antioxidant GSH and suppressing the formation of TBARS in cardiac tissue.

The presence of antioxidant potential and polyphenolic compounds in EXS and EAXS may be responsible for the significant decrease in the LDH,CKMB,TBARS and significant increase in GSH levels. Hence the result suggested that the cardioprotective effect against doxorubicin induced cardiotoxicity at 100mg/kg & 200mg/kg of EXS and 10mg/kg of EAXS.

PART II

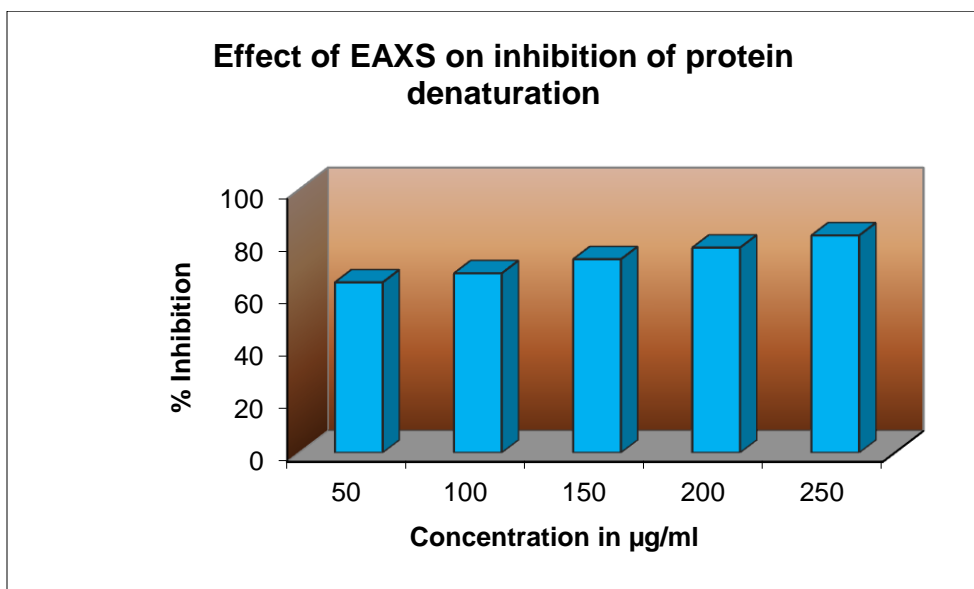
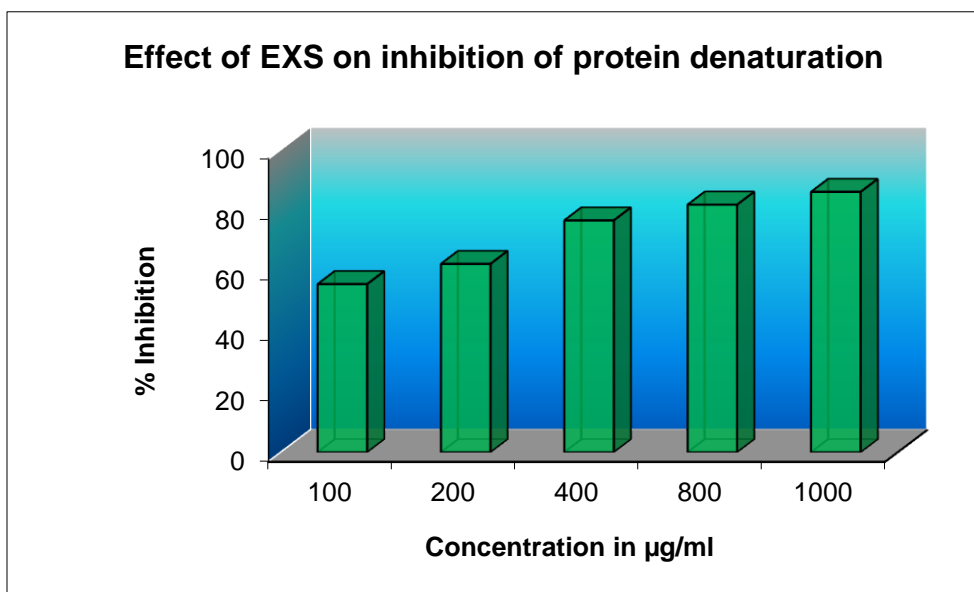
This part of the pharmacological study deals with the in vitro antiarthritic activity of both EXS and EAXS

The principle involved is the inhibition of protein denaturation. Denaturation of protein was found to be one of the cause of rheumatoid arthritis. In rheumatoid arthritis the autoantigen production may be due to denaturation of protein. The mechanism of denaturation involves the alteration of electrostatic hydrogen, hydrophobic and disulphide bonding.

The protein used in this study is bovine serum albumin. Denaturation of protein is carried out by heating. The phenomenon of denaturation of protein by heat was determined by Mizushima, 1996. The aim of this activity is to inhibit denaturation and to exhibit protective effect against rheumatoid arthritis. Various concentrations of EXS (100, 200, 400, 800, 1000 µg/ml) and EAXS (50,100,150,200,250 µg/ml) were subjected to invitro antiarthritic activity. The percentage inhibition of protein denaturation by EXS and EAXS were compared with standard drug (Diclofenac sodium 250 µg/ml). The results were presented in the table.

It has been reported that anti arthritic activity may be due to the presence of phenolic compounds .The inhibition of protein denaturation by EXS and EAXS were may be due to the presence of flavanoids, tannins. The EXS showed 85.98 % inhibition at 1000 µg/ml and EAXS showed 82.56% inhibition at 250µg/ml when compared to standard drug Diclofenac sodium 92%(250µg/ml)

Fig 20 Effect of EXS and EAXS on Inhibition of Protein Denaturaion



PART III

INVITRO ANTI-BACTERIAL ACTIVITY

This part of the pharmacological study deals with the anti-bacterial activity against causative organisms for rheumatic fever and endocarditis.

Both EXS and EAXS were subjected to anti-bacterial activity against *Streptococcus pyrogenes*, *Streptococcus viridians* and *Enterococcus faecalis*.

Zone of inhibition of EXS (6mg) was found to be, *Streptococcus pyrogenes*,(12mm) *Streptococcus viridians*(12mm) and *Enterococcus faecalis*(12mm) compared to penicillin. Zone of inhibition of EAXS was found to be, *Enterococcus faecalis*(22mm) *Streptococcus pyrogenes*,(20mm) and *Streptococcus viridians*(22mm), which indicate that this fraction has exerted significant anti-bacterial activity against all the three bacterial pathogens than EXS when compared to the standard drug penicillin.

FIG : 1.1 T.S OF LEAF THROUGH MIDRIB

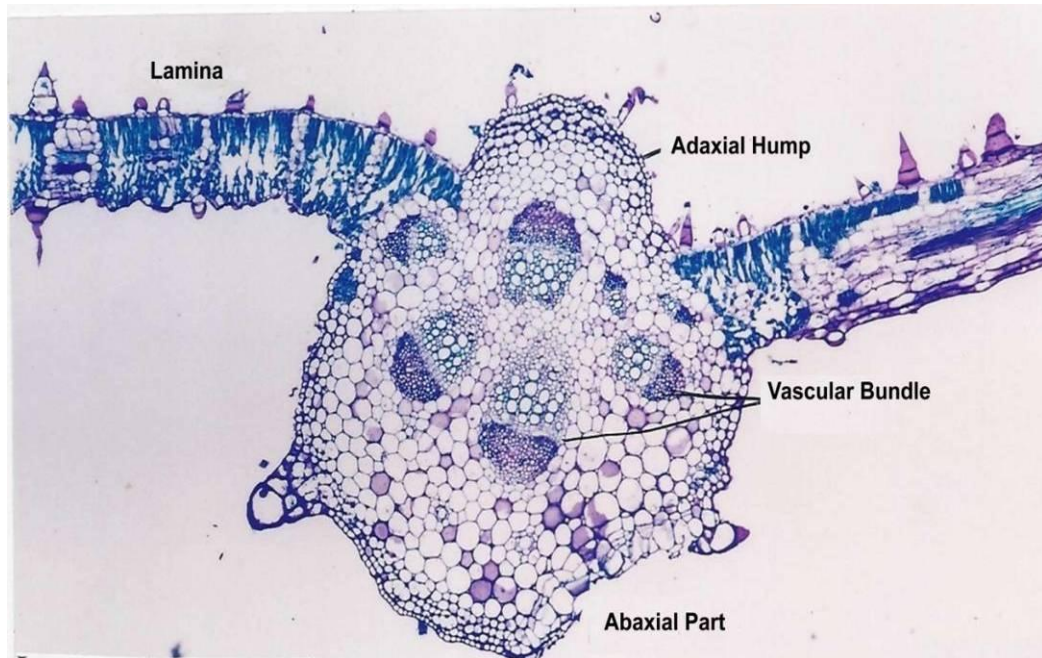


FIG : 1.2 T.S OF MIDRID ENLARGED

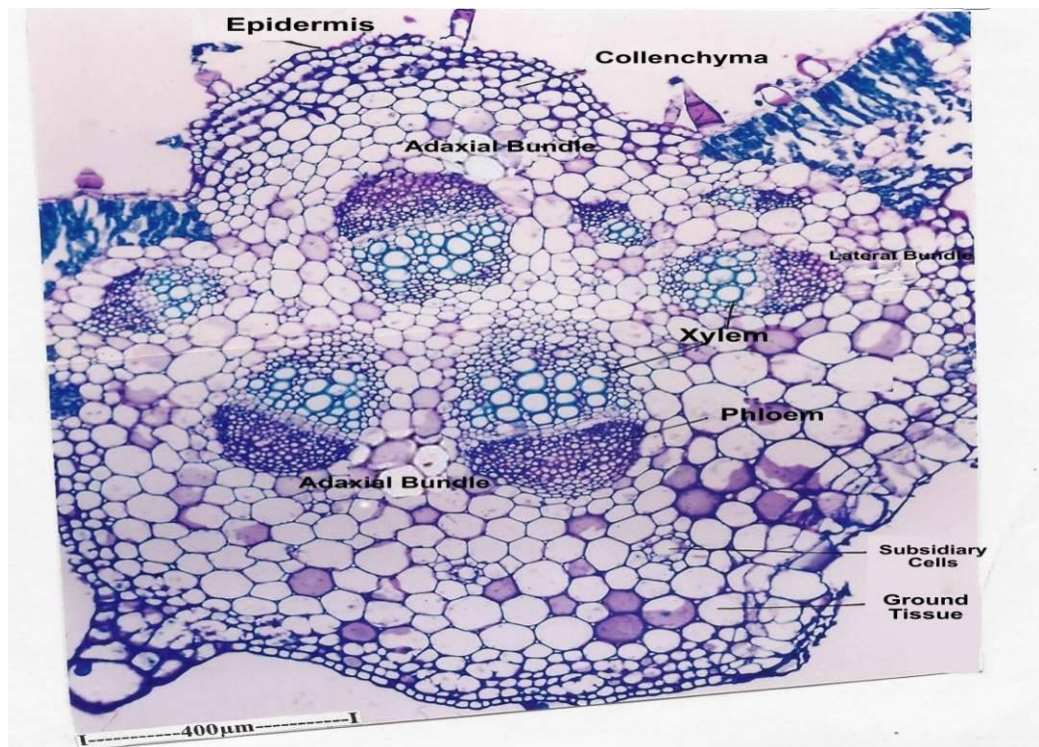


FIG : 2.1 T.S OF LAMINA SHOWING EPIDERMAL TRICHOMES AND MESOPHYLL TISSUE

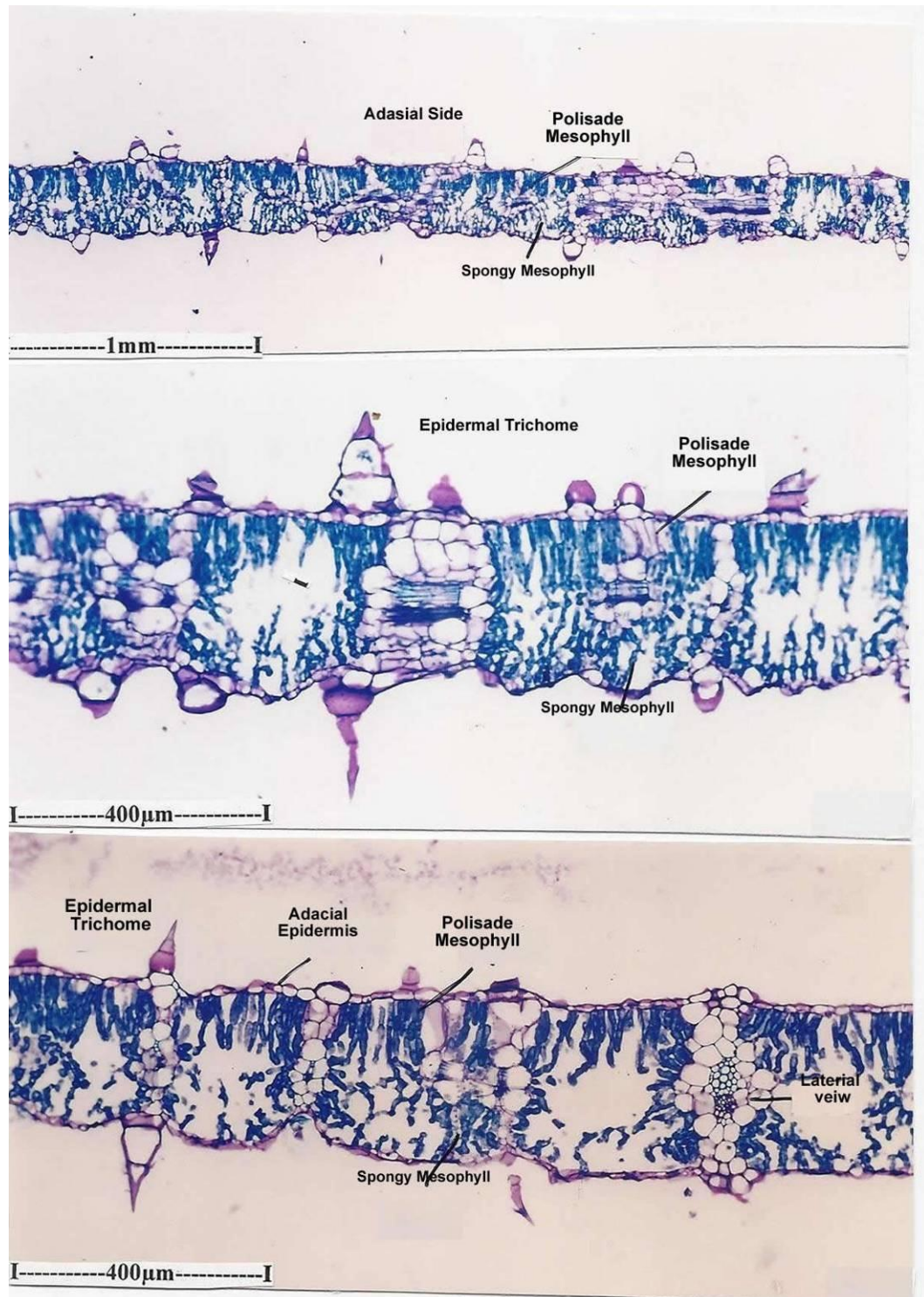


FIG : 3.1 T.S OF LAMINA SHOWING ENLARGED VIEW OF TRICHOMES

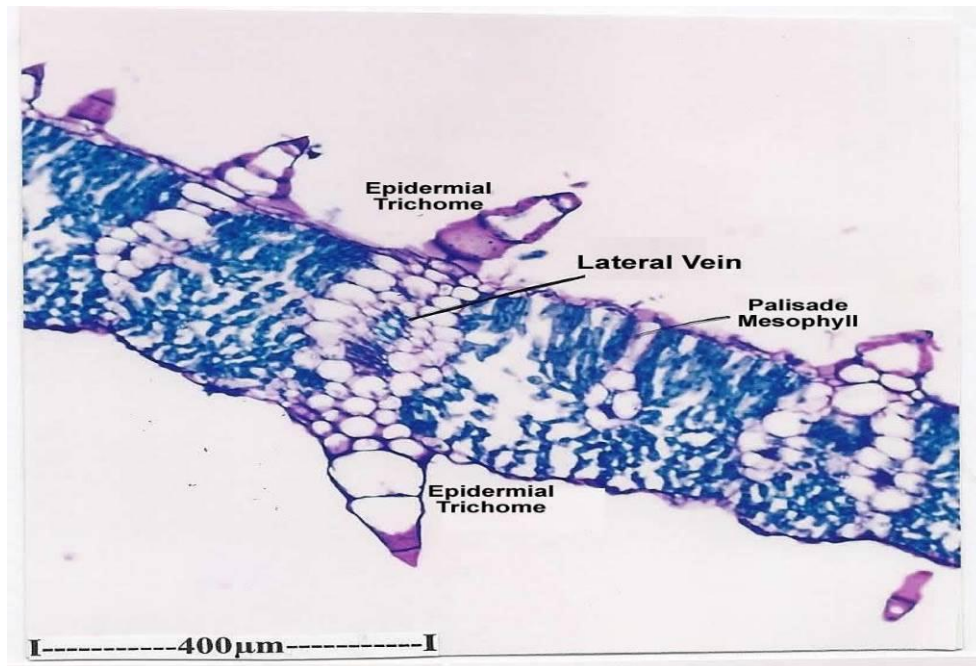
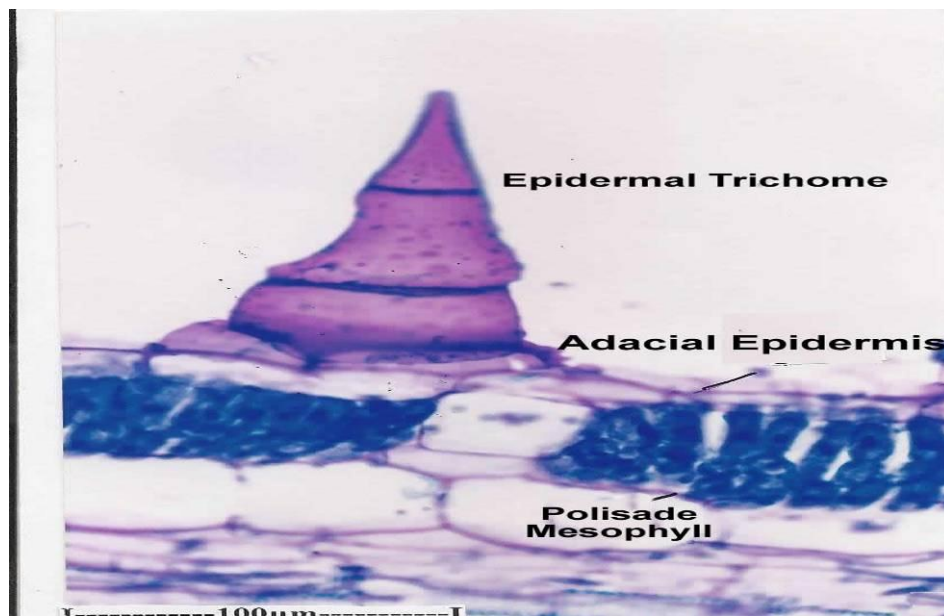
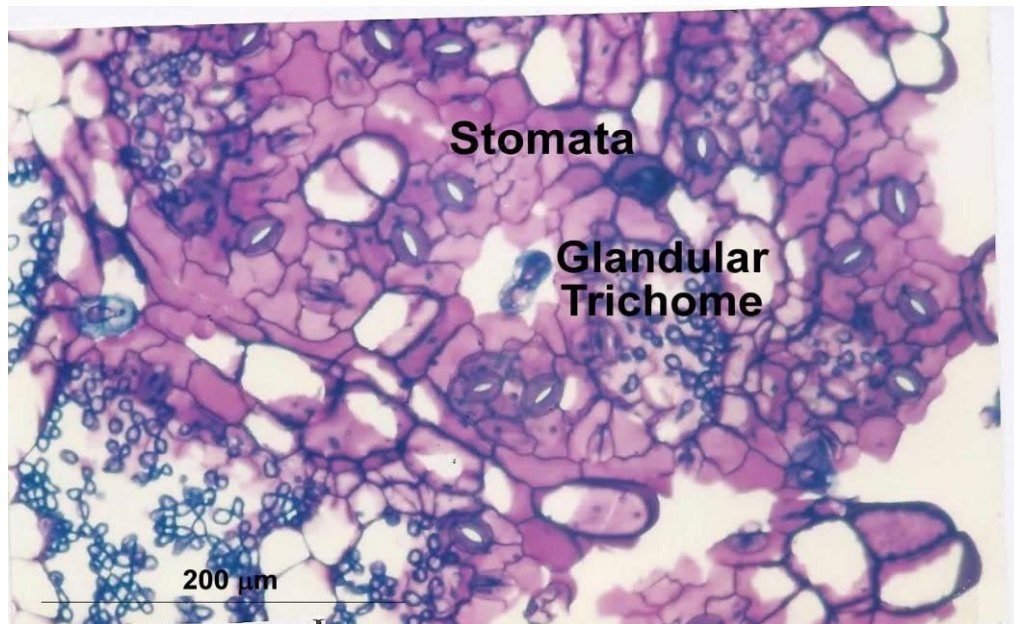


FIG : 3.2 T.S OF LAMINA SHOWING PORTION ENLARGED VIEW OF TRICHOMES



**FIG : 4.1 PARADERMAL SECTION OF THE LAMINA
SHOWING THE STOMATA AND GLANDULAR
TRICHOMES**



**FIG : 4.2 PARADERMAL SECTION OF THE LAMINA
SHOWING THE STOMATA AND GLANDULAR
TRICHOMES**

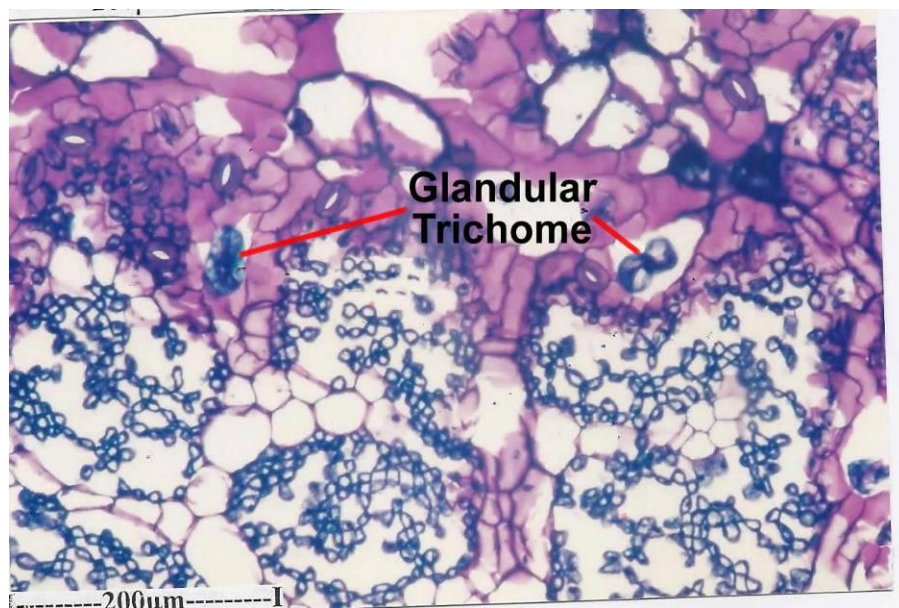


FIG : 5.1 SURFACE VIEW OF STOMATA

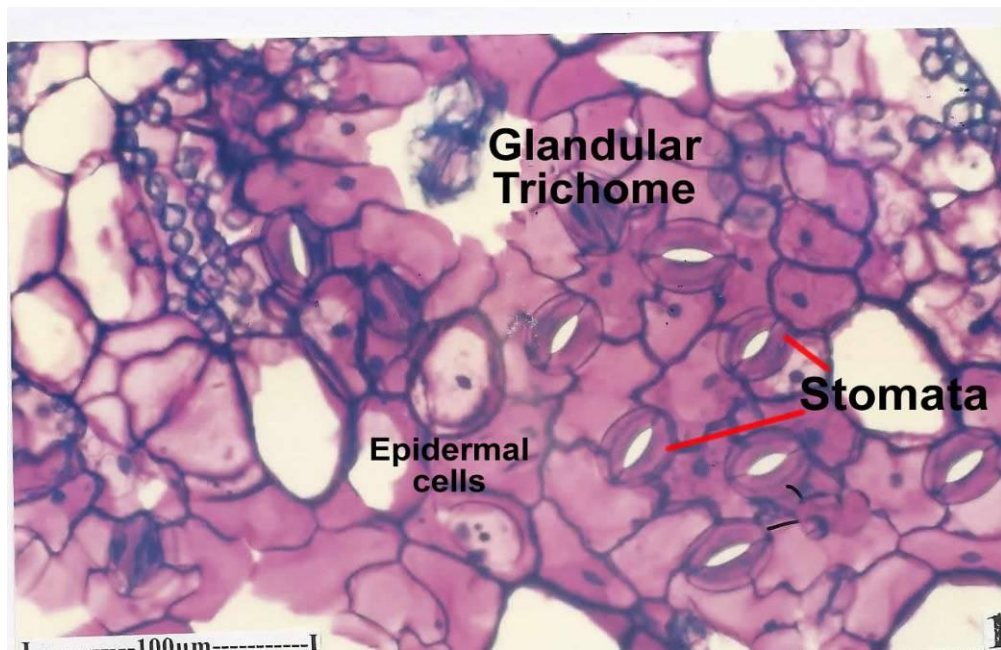
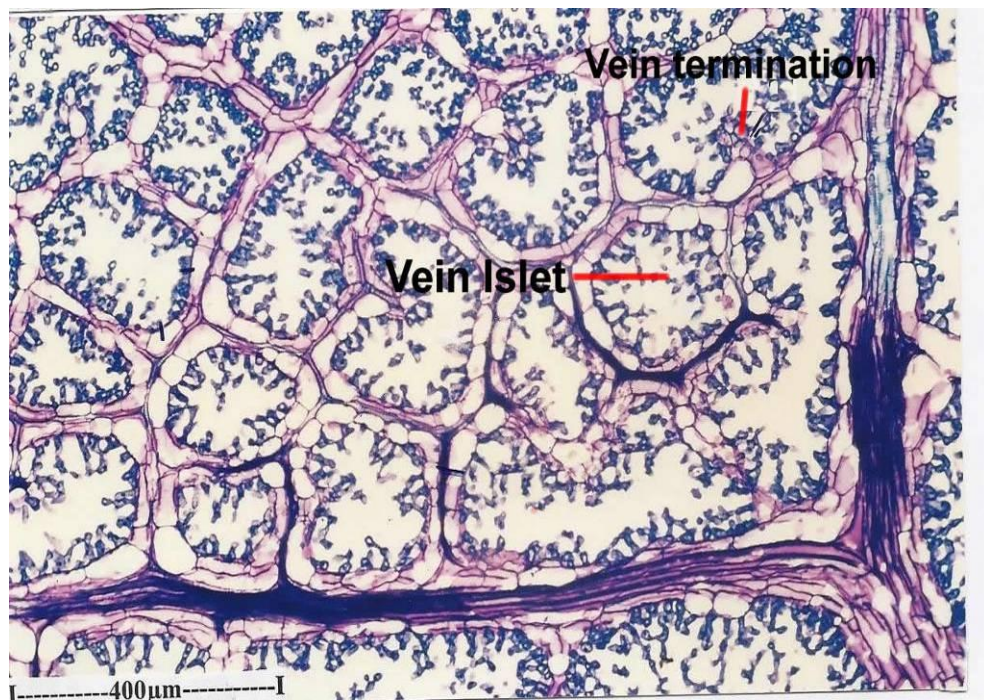


FIG : 5.2 PARADERMAL SECTION SHOWING VENATION OF THE LAMINA



**FIG : 6.1 VENATION AND GLANDULAR TRICHOMES
IN SURFACE VIEW**

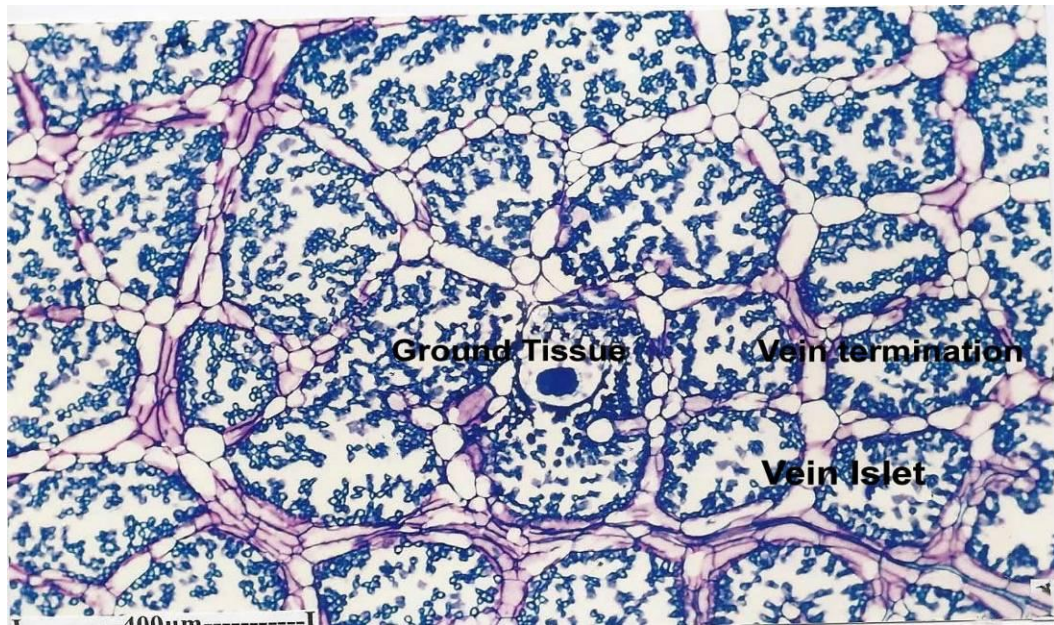


FIG : 6.2 GLANDULAR TRICHOMES ENLARGED

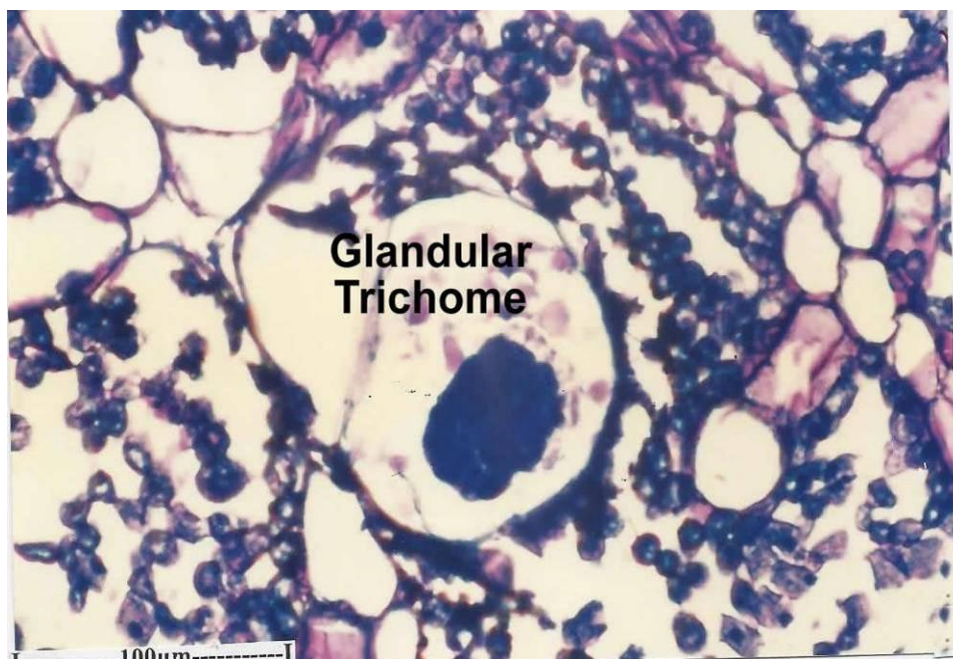


FIG : 7.1,2 T.S OF BASAL PART OF PETIOLE

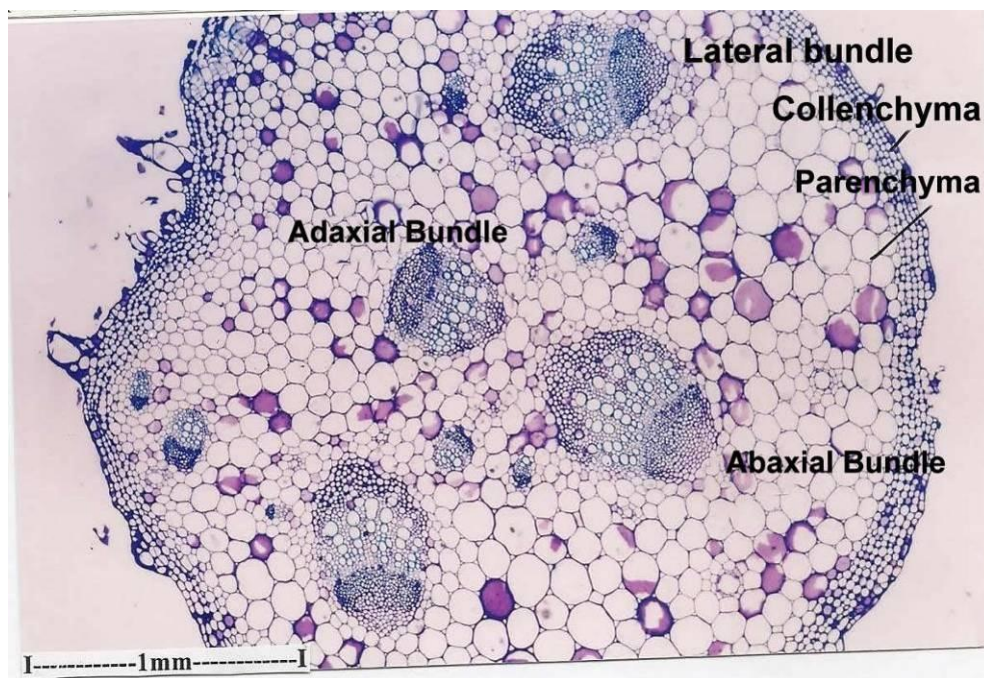
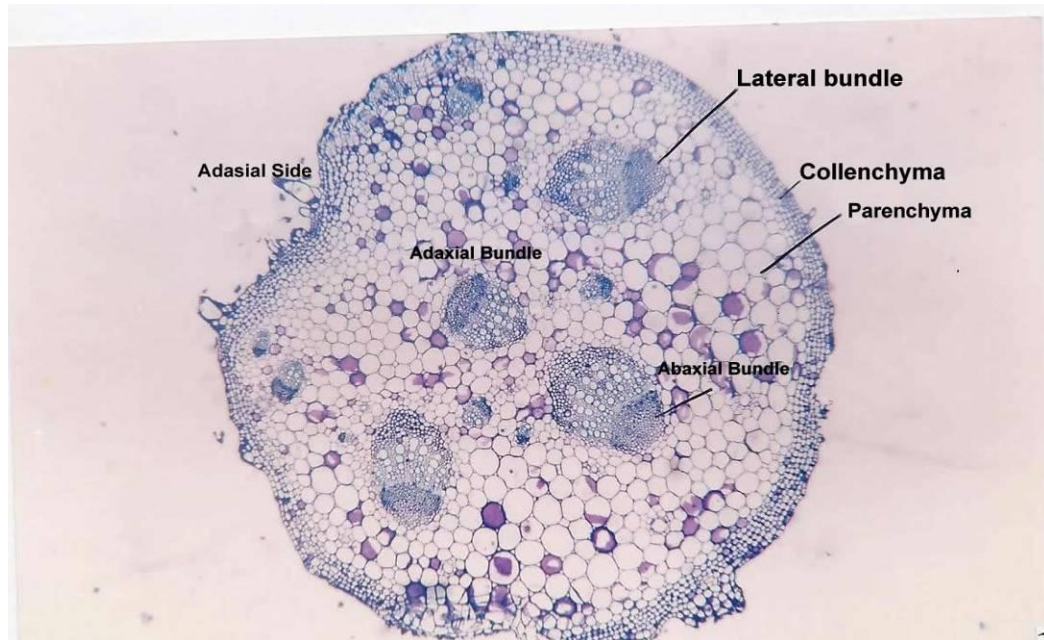


FIG : 8.1 T.S OF MIDDLE PART OF PETIOLE

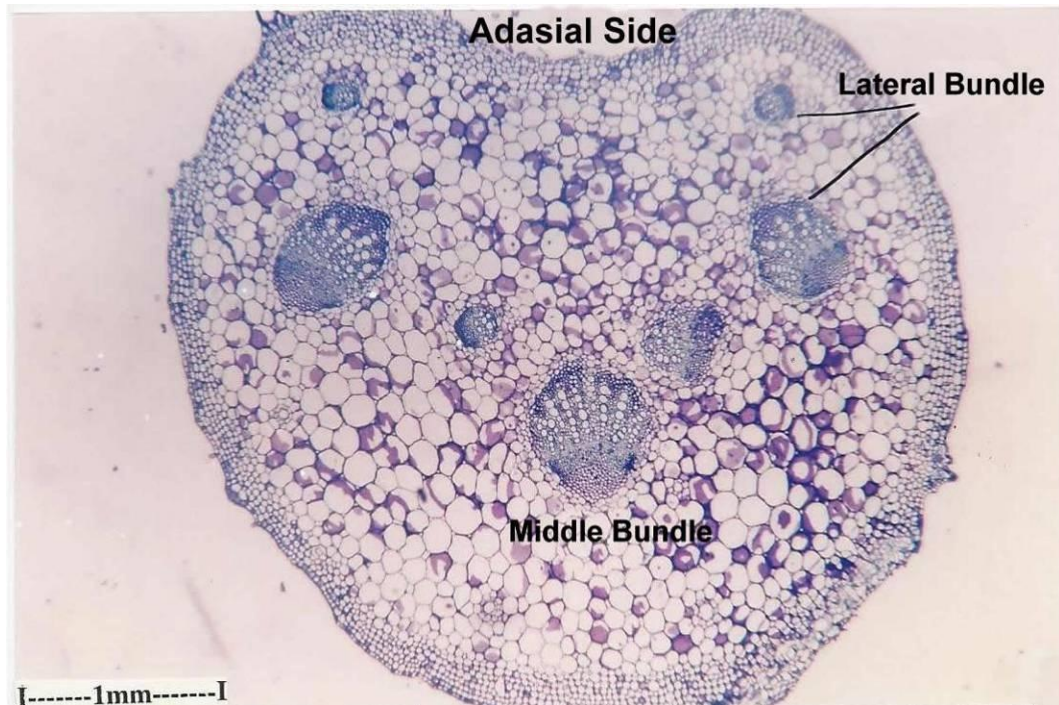


FIG : 8.2 ONE SECTOR ENLARGE

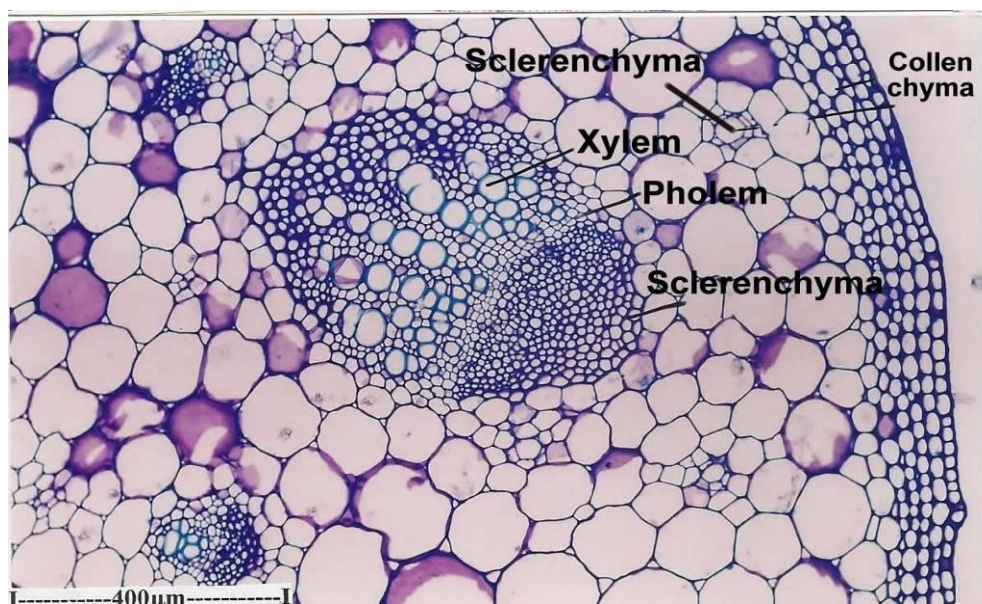


FIG : 9.1 T.S. OF TERMINAL PART OF PETIOLE

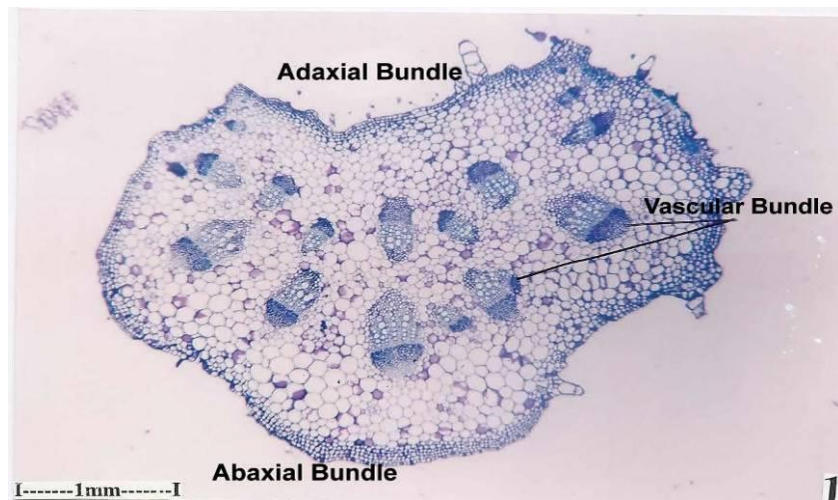


FIG : 9.2 A SECTION ENLARGED

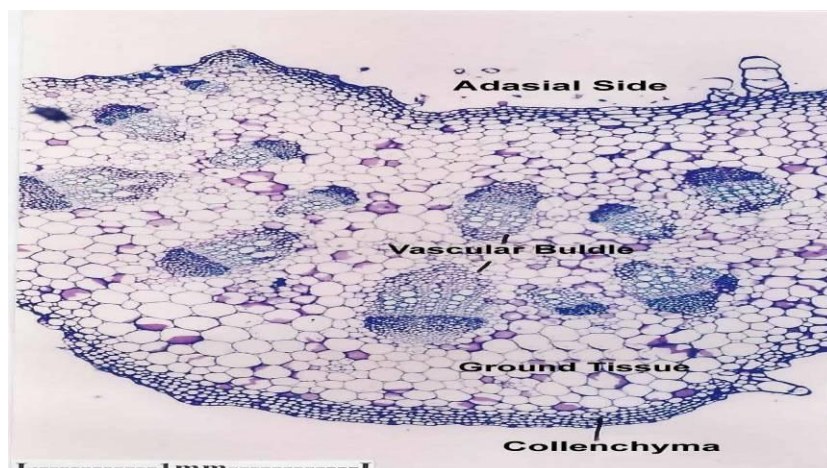


FIG : 9.3 A VASCULAR BUNDLE ENLARGED



FIG : 10.1 FRAGMENTS OF THE LEAF IN THE POWDER SHOWING VENATION TYPE

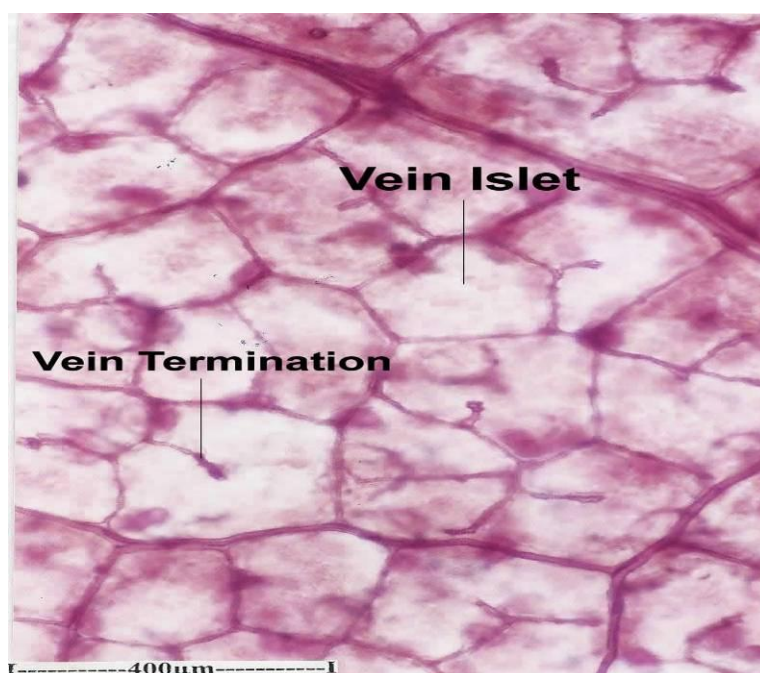
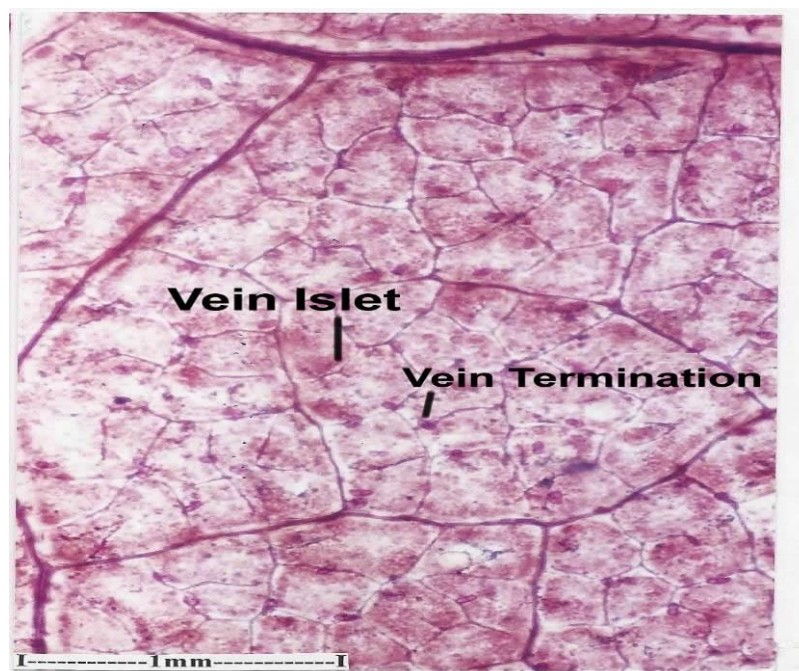


FIG : 10.2 FRAGMENTS OF THE LEAF IN THE POWDER SHOWING VENATION TYPE



**FIG : 10.3 EPIDERMAL NON GLANDULAR
TRICHOMES**

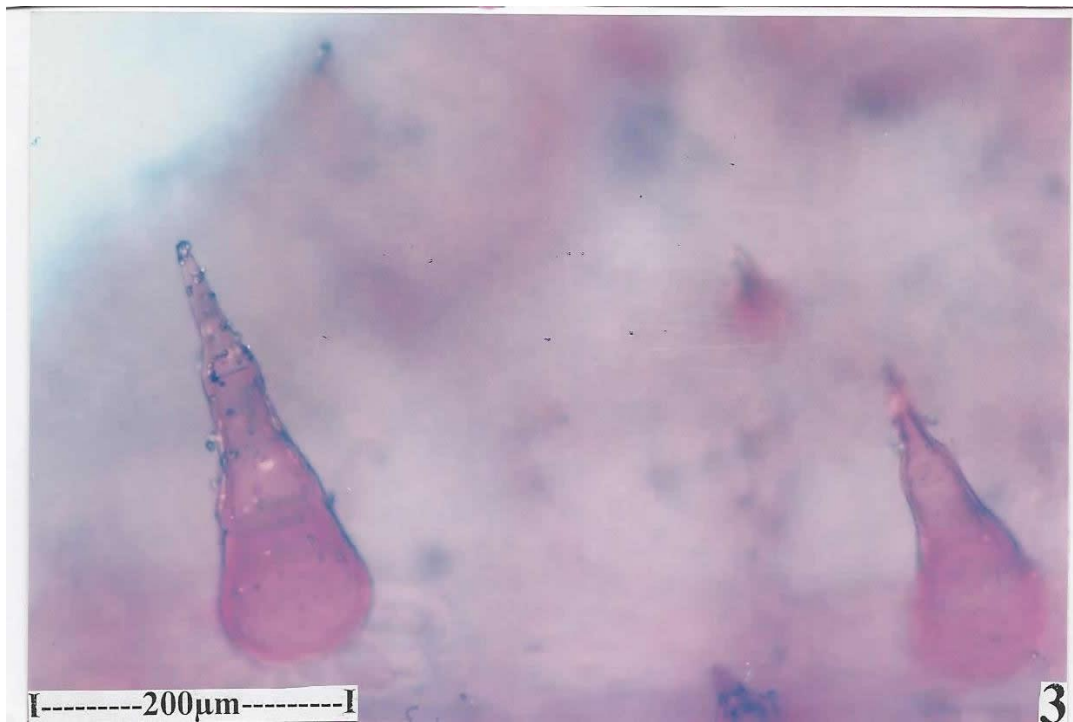


FIG: 12 FLOWER OF XANTHIUM STRUMARIUM



FIG 13 FRUITS OF XANTHIUM STRUMARIUM



**FIG 11 HABITAT OF XANTHIUM
STRUMARIUM**



FIG 14 LEAVES OF XANTHIUM STRUMARIUM
DORSAL VIEW

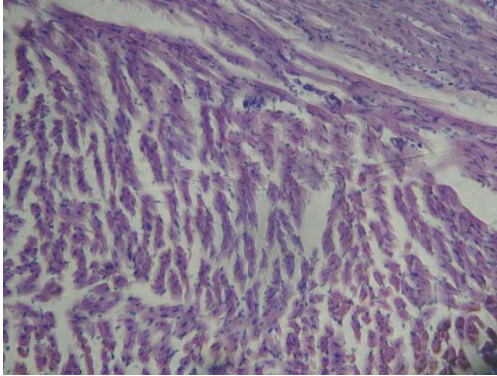


VENTRAL VIEW

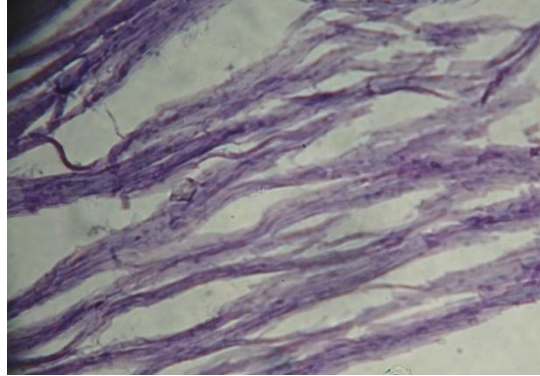


HISTOPATHOLOGICAL STUDIES OF CARDIAC TISSUE

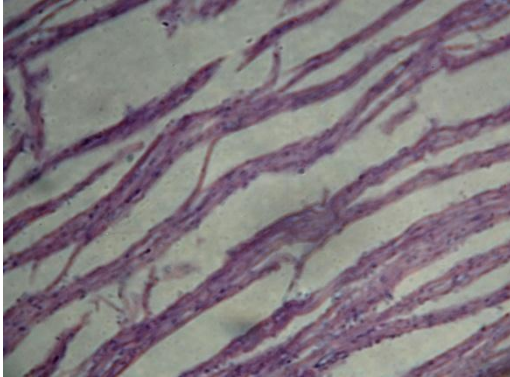
Normal Group



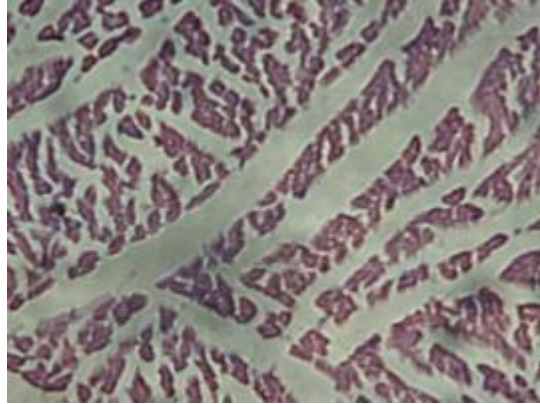
Doxorubicin Treated Group



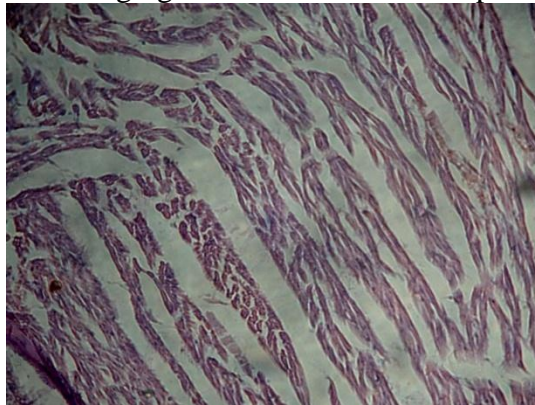
100 mg/kg of EXS treated Group



200 mg/kg of EXS treated Group



10 mg/kg of EAXS treated Group

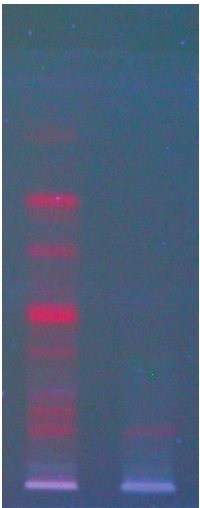


VISUALIZATION

@ 254nm



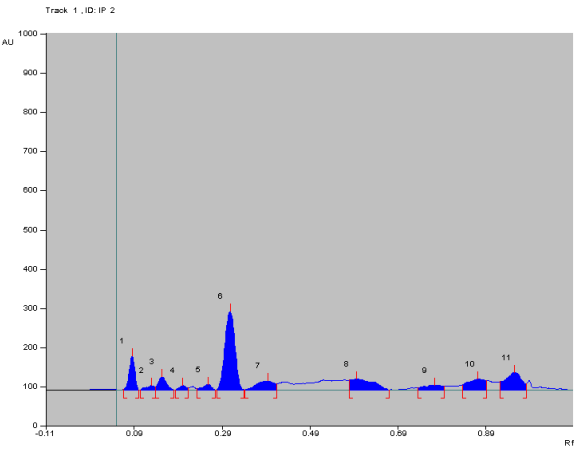
@ 366nm



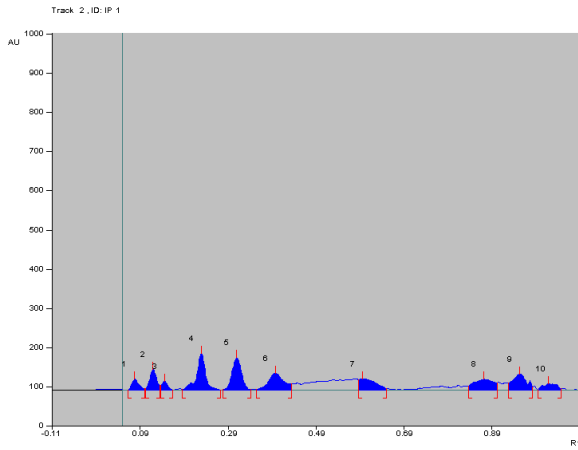
PEAK DISPLAY

@ 254nm

Track 1

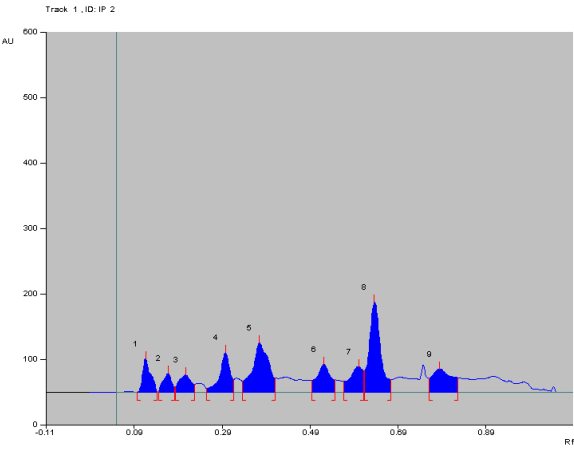


Track 2

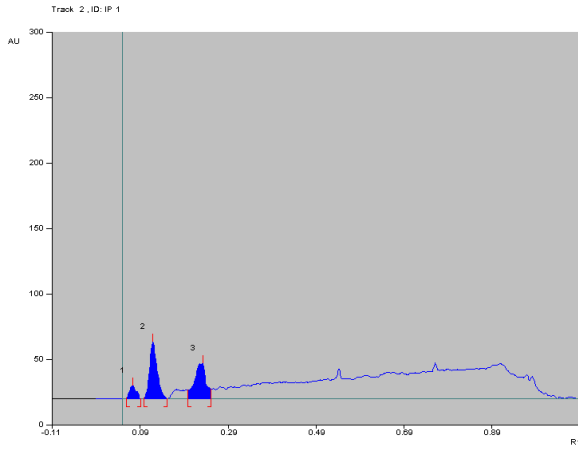


@ 366nm

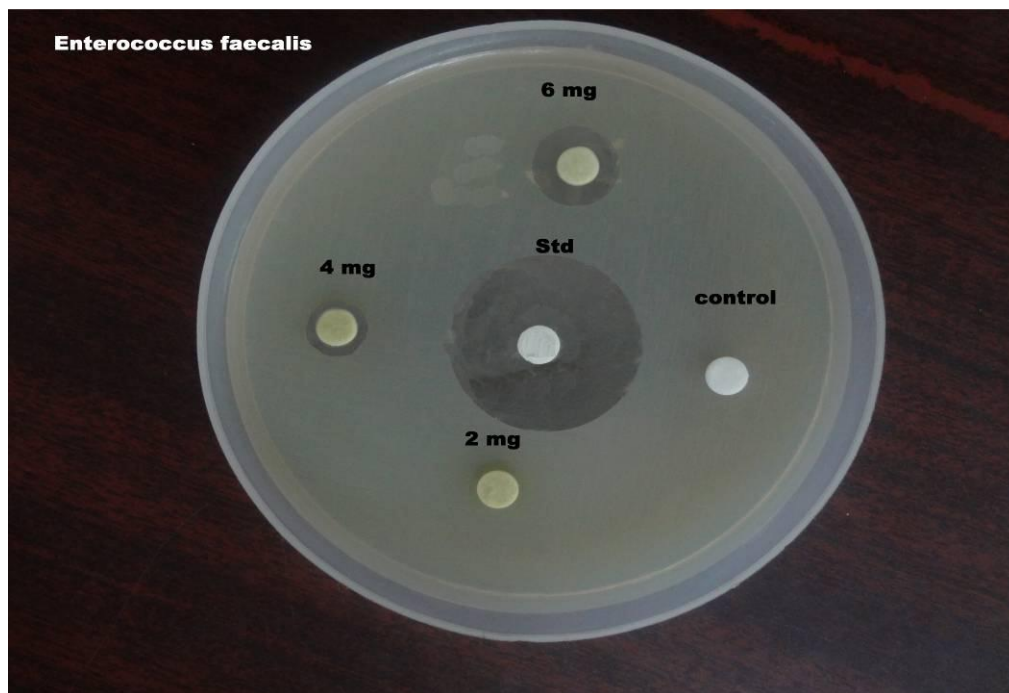
Track 1



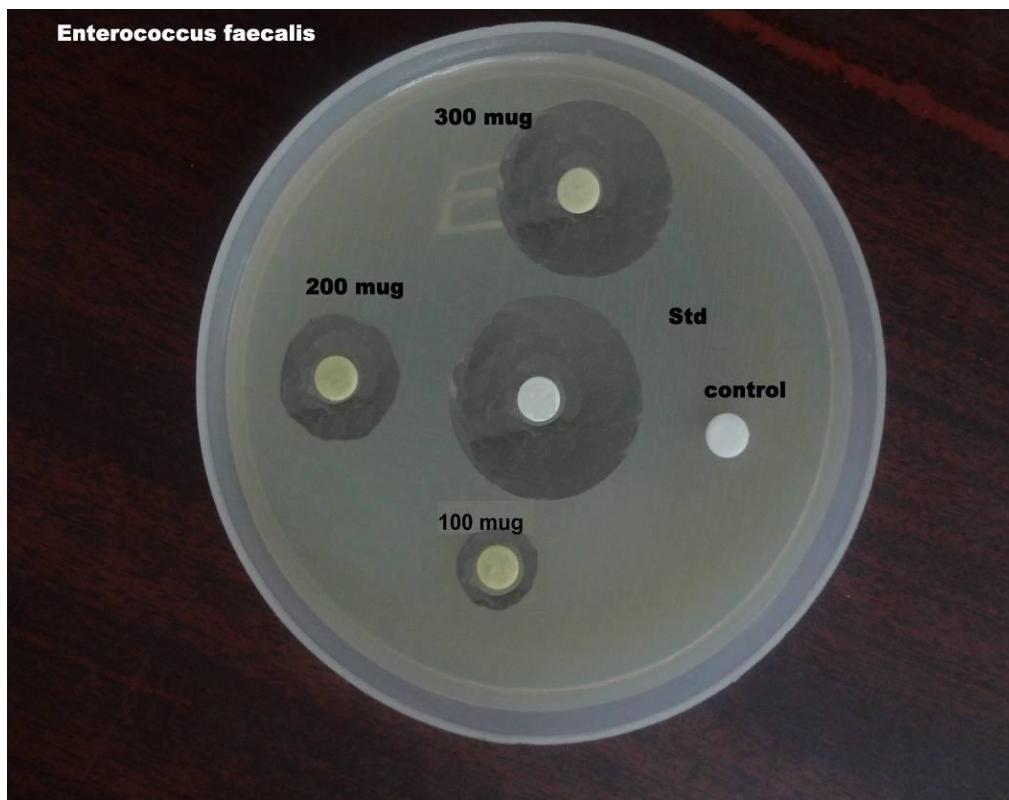
Track 2



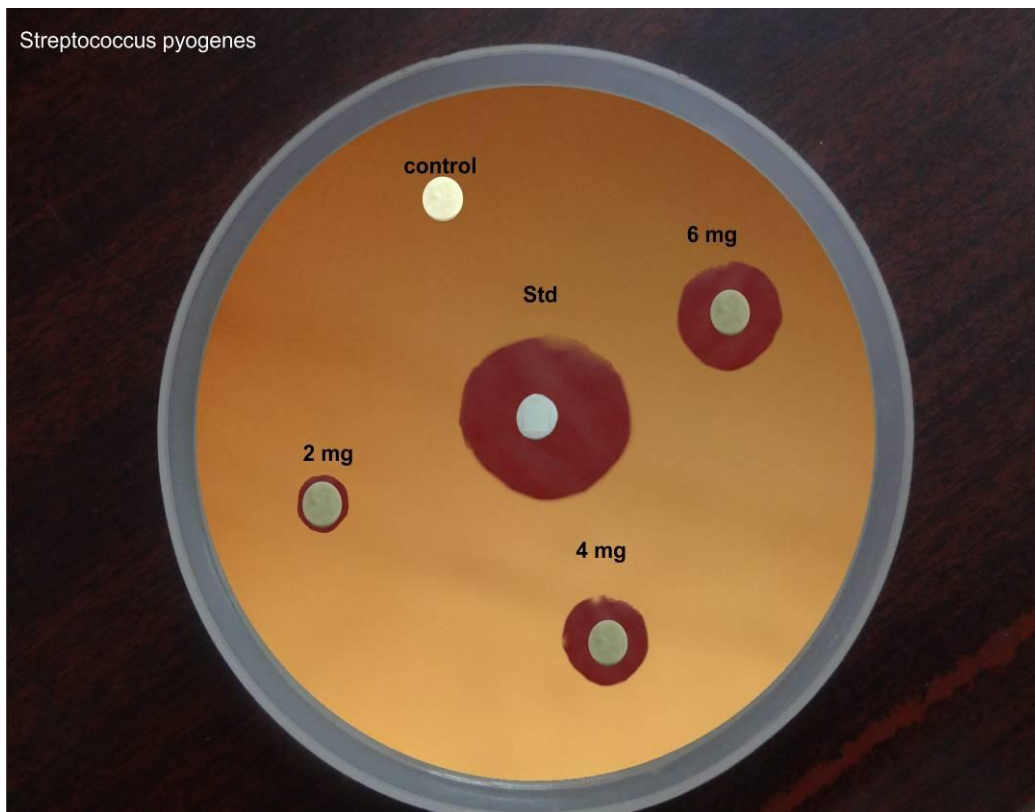
EFFECT OF EXS AGAINST ENTEROCOCCUS FAECALIS



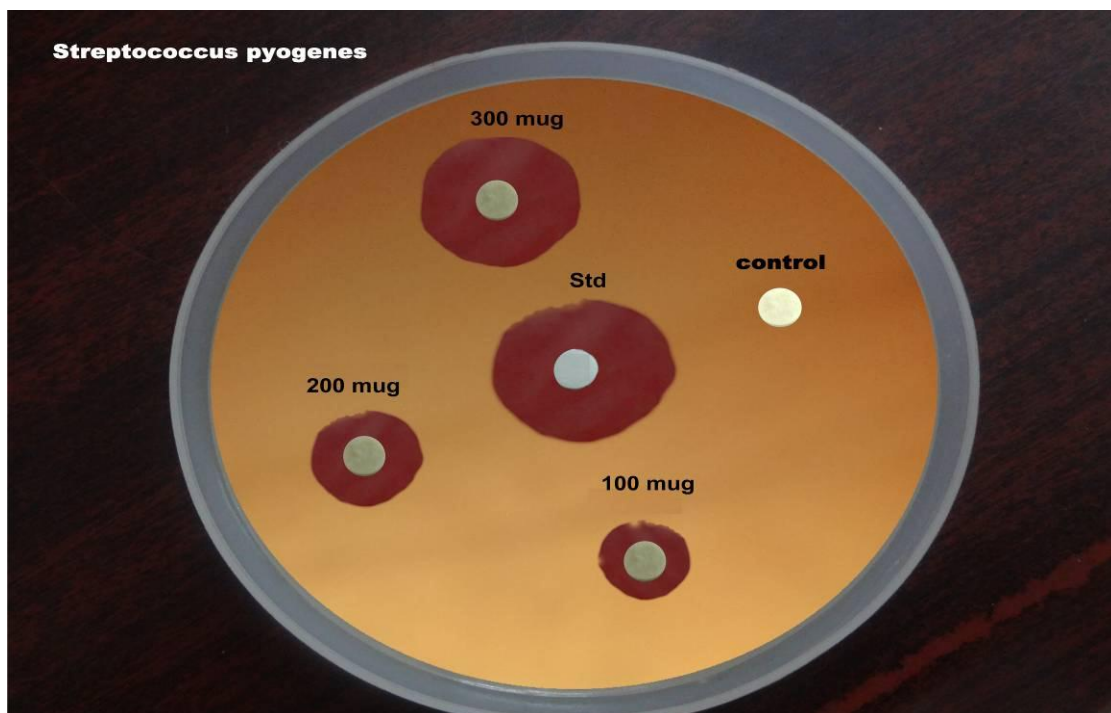
EFFECT OF EAXS AGAINST ENTEROCOCCUS FAECALIS



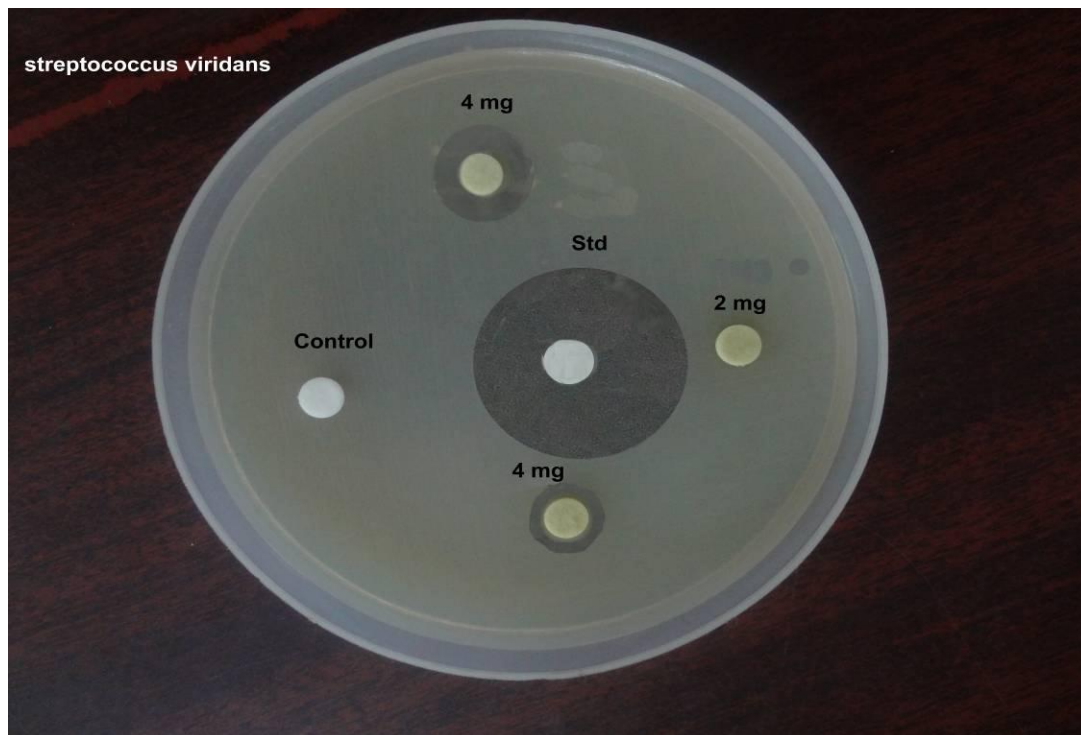
EFFECT OF EXS AGAINST STREPTOCOCCUS PYROGENES



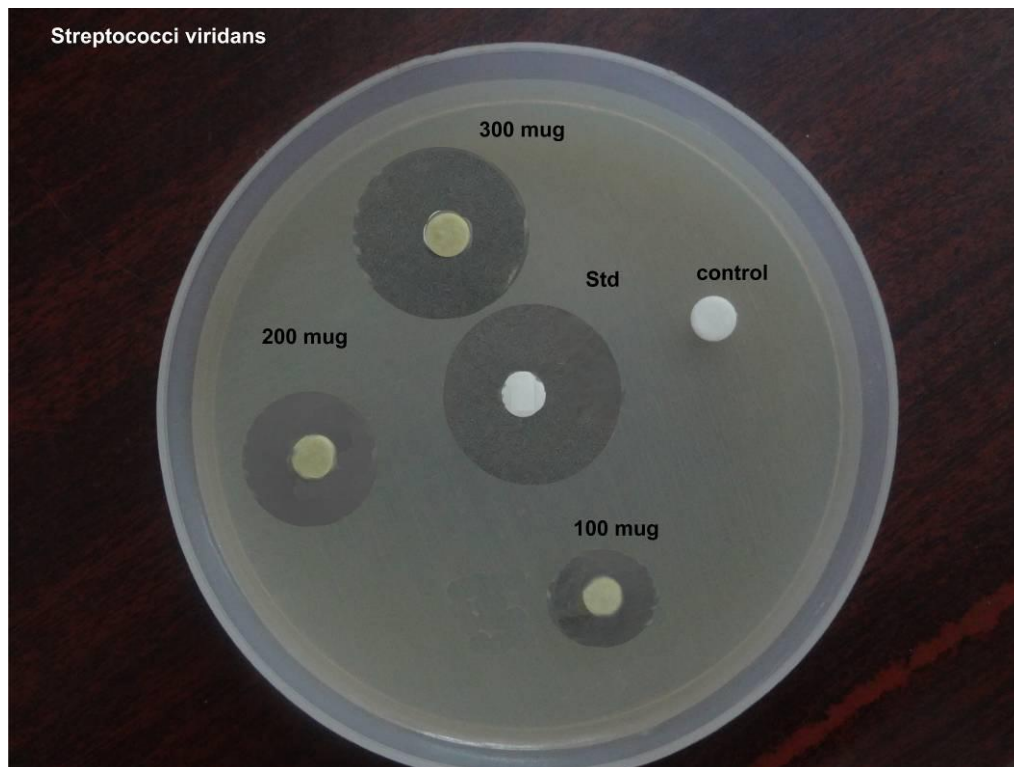
EFFECT OF EAXS AGAINST STREPTOCOCCUS PYROGENES



EFFECT OF EXS AGAINST STREPTOCOCCUS VIRIDANS



EFFECT OF EAXS AGAINST STREPTOCOCCUS VIRIDANS



Xanthium strumarium, linn



CHAPTER-8

CONCLUSION

There are many unknown plants with high medicinal value still have not been recognised their importance. They have not been brought to the light of scientific world. This dissertation covers pharmacognostical, phytochemical and pharmacological studies on the commonly available medicinal plant *Xanthium strumarium* belonging to the family asteracea.

Pharmacognostical parameters have been determined on the leaves inorder to substantiate and identify the plant for future work.

Preliminary phytochemical screening on the leaves of *X.strumarium* confirms the presence of flavanoids, phenols, tannins, sterols, alkaloids, carbohydrates and saponins.

Preparation of extract and isolation of phenolic rich fractions were carried out in this study.

Determination of total phenolics(221.9mg/g), total tannins(327mg/g) and total flavanoid content(23.03mg/g) confirms the significant concentration of these phytoconstituents in the ethanolic extract of *X.strumarium*. Total phenolic content was estimated for all the phenolic rich fractions which indicate that ethylacetate fraction have shown significant phenolic content.. Evaluation of phytoconstituents were performed by TLC and HPTLC studies on EXS and EAXS

Pharmacological screening confirms,

- Both EXS and EAXS have exhibited radical scavenging activity by DPPH assay and EXS has shown radical scavenging activity by nitric oxide

scavenging and it also exerted significant antioxidant potential by phosphomolybdenum method.

- Significant cardioprotective activity at 100mg/kg of EXS ($p<0.001$), 200 mg/kg of EXS ($p<0.001$) and 10 mg/kg EAXS ($p<0.001$) against Doxorubicin induced cardiotoxicity in rats and the *invivo* antioxidant activity of these extracts may be ascribed for this cardioprotective activity which is confirmed by elevation of GSH ($p<0.001$) and reduction of TBARS ($p<0.001$) in the tissue parameters of the treated animals.
- 1000 μ g/ml of EXS and 250 μ g/ml of EAXS showed significant anti – arthritic activity by inhibition of protein denaturation.
- Significant anti-bacterial activity exhibited by EAXS (300mcg) against *Streptococcus pyrogenes* which is a causative organism for rheumatic fever. *Streptococci viridians* and *Enterococci faecalis* also sensitive to this extracts and these pathogens cause endocarditis. Anti-bacterial activity of this extract was comparable with standard drug penicillin.

It was evident from the phytochemical studies of this plant, that substantial amount of phenols, tannins and flavanoids were present in these extracts which exhibited significant *invitro* antioxidant activity.

Heart failure is a burgeoning problem world wide, with more than 20 million people were affected. The overall prevalence of HF in adult population is 2% and the risk rises with age affecting 6 – 10 % of people over 65 years of age. Cardiomyopathy may be due to viruses, amyloidosis, diabetes, thyroid diseases, medications such as chemotherapy drugs (Doxorubicin,

Donorubicin,etc). Though many synthetic cardioprotective agents are available, herbal drugs are preferred for their safety and efficacy.

Cardiotoxicity was induced in the animal model using Doxorubicin (antineoplastic agent). Doxorubicin cause oxidative stress leads to cardiac tissue damage at 15mg/kg i.p in rats. Cardioprotective activity of these extracts may be due to the presence of phenolic phytoconstituents by virtue of their antioxidant and radical scavenging properties.

Rheumatoid arthritis being a common inflammatory disease affects about 1% of the adult population worldwide. It occurs in an immunogenitically predisposed individuals. Protein denaturation was found to be one of the cause of R.A. Both EXS and EAXS have shown significant anti-arthritic activity and the phenolic constituents may be responsible for this activity.

These extracts have also shown significant anti-bacterial activity against the pathogens caused for rheumatic fever and endocarditis. Phenolic phytoconstituents may be responsible for this activity.

Phenolic secondary metabolite responsible for invitro antioxidant, cardioprotective activity, anti-arthritic activity and anti bacterial activity may be isolated and evaluated for further studies.

It may also serve as a lead molecule to synthesise various semisynthetic drugs to treat various life threatening diseases like CHF, RA, rheumatic fever caused by *Streptococcus pyrogenes* and endocarditis caused by *Enterococcus faecalis*, *Streptococci viridians*.